Dovi, S.
09/044696

(FILE 'USPAT' ENTERED AT 15:31:41 ON 27 JUL 1999)
L1 1085 SEA (ADP(W)RIBOSYLAT? OR CHOLER? OR PERTUSS? OR COLI)(3W) TOXIN# OR (CT OR PT OR LT)(10A)TOXIN#
L2 9 SEA L1 AND ((DETOX? OR DE(W)TOX?)(5A)(MUTAT? OR MUTAGEN? OR MUTANT#))

=> d 1-9 .bevpat

US PAT NO: 5,856,122 [IMAGE AVAILABLE] L2: 1 of 9
TITLE: Modification of **pertussis toxin**
DATE ISSUED: Jan. 5, 1999
INVENTOR: Randy J. Read, Edmonton, Canada
Penelope E. Stein, Edmonton, Canada
Stephen A. Cockle, Richmond Hill, Canada
Raymond P. Oomen, Tottenham, Canada
Sheena Loosmore, Aurora, Canada
Michel H. Klein, Willowdale, Canada
Glen D. Armstrong, Edmonton, Canada
Bart Hazes, Edmonton, Canada
SEARCH-FLD: 435/15, 69.1

ABSTRACT:
The three-dimensional structure of crystalline pertussis holotoxin (PT) has been determined by X-ray crystallography. Crystal structures have also been determined for complexes of **pertussis toxin** with molecules relevant to the biological activity of **PT**. These three-dimensional structures were analyzed to identify functional amino acids appropriate for modification to alter the biological properties of PT. Similar procedures may be used to predict amino acids which contribute to the toxicity of the holotoxin, to produce immunoprotective, genetically-detoxified analogs of **pertussis toxin**.

US PAT NO: 5,849,530 [IMAGE AVAILABLE] L2: 2 of 9
TITLE: Manipulation of gene copy number in bordetella
DATE ISSUED: Dec. 15, 1998
INVENTOR: Sheena Loosmore, 70 Crawford Rose Drive, Aurora, Ontario, Canada, L4G 4R4
Gavin Zealey, 348 Charlton Avenue, Thornhill, Ontario, Canada, L4J 6H7
Reza Yacoob, 2354 Old Pheasant Road, Mississauga, Ontario, Canada, L5A 2S1
Michel Klein, 16 Munro Boulevard, Willowdale, Ontario, Canada, M2P 1B9
SEARCH-FLD: 435/69.3, 69.1, 172.3, 243, 252.3, 320.1; 424/234.1, 235.1, 240.1, 184.1, 192.1; 536/23.1, 23.2, 23.7

ABSTRACT:
A protein expression levels from Bordetella strains, particularly Bordetella pertussis, are altered by genetic modification to a natural Bordetella strain whereby one or more of the natural genes, particularly

including the TOX, FHA, CYA and PRN genes, is deleted from the genome of the natural strain and one or more of the natural genes or a genetic **mutation** thereof, particularly a genetically-**detoxified** TOX* gene, or a hybrid gene, is inserted into the genome of the natural strain to provide at least two copies of one or more of the natural genes or genetic mutation thereof or hybrid gene, singly or in tandem. The altered genotype Bordetella strain is useful in producing whole-cell or defined component vaccines against Bordetella, particularly whooping cough, which may be employed in combination with other vaccines.

US PAT NO: 5,439,810 [IMAGE AVAILABLE] L2: 3 of 9
TITLE: Manipulation of gene copy number in bordetella
DATE ISSUED: Aug. 8, 1995
INVENTOR: Sheena Loosmore, Aurora, Canada
Gavin Zealey, Thornhill, Canada
Reza Yacoob, Mississauga, Canada
Michel Klein, Willowdale, Canada
SEARCH-FLD: 424/88, 92, 234.1, 235.1, 240.1; 435/69.1, 172.3, 243, 69.3, 320.1, 252.3; 935/38; 536/27, 23.1, 23.2, 23.7

ABSTRACT:
Protein expression levels from Bordetella strains, particularly Bordetella pertussis, are altered by genetic modification to a natural Bordetella strain whereby one or more of the natural genes, particularly including the TOX, FHA, CYA and PRN genes, is deleted from the genome of the natural strain and one or more of the natural genes or a genetic **mutation** thereof, particularly a genetically-**detoxified** TOX* gene, or a hybrid gene, is inserted into the genome of the natural strain to provide at least two copies of one or more of the natural genes or genetic mutation thereof or hybrid gene, singly or in tandem. The altered genotype Bordetella strain is useful in producing whole-cell or defined component vaccines against Bordetella, particularly whooping cough, which may be employed in combination with other vaccines.

US PAT NO: 5,433,945 [IMAGE AVAILABLE] L2: 4 of 9
TITLE: Immunoprotective genetically-**detoxified mutants** of **pertussis toxin**
DATE ISSUED: Jul. 18, 1995
INVENTOR: Michel H. Klein, Willowdale, Canada
Heather A. Boux, Aurora, Canada
Stephen A. Cockle, Richmond Hill, Canada
Sheena M. Loosmore, Aurora, Canada
Gavin R. Zealey, Concord, Canada
SEARCH-FLD: 424/88, 92, 93, 94; 435/252.3; 530/350, 351, 387, 388, 403, 405, 406

ABSTRACT:
A new method is described for the preparation of a safe, immunogenic and efficacious vaccine for protection against the disease pertussis. In development of this vaccine, specific functional sites of **pertussis toxin** have been identified, and using this information, defined

mutant holotoxins have been produced by site directed mutagenesis of the toxin gene. A number of these holotoxin analogues are detoxified, retain an immunodominant S1 epitope, are immunogenic and are protective in the standard pertussis vaccine potency test in mice.

US PAT NO: 5,358,868 [IMAGE AVAILABLE] L2: 5 of 9
TITLE: Genetic detoxification of **pertussis toxin**
DATE ISSUED: Oct. 25, 1994
INVENTOR: Michel H. Klein, Willowdale, Canada
Heather A. Boux, Aurora, Canada
Stephen A. Cockle, Richmond Hill, Canada
Sheena M. Loosmore, Aurora, Canada
Gavin R. Zealey, Concord, Canada
SEARCH-FLD: 435/69.1, 69.3, 252.1, 172.1, 172.2, 172.3, 243; 536/27, 23.5; 935/10, 11, 12, 65; 530/324, 350

ABSTRACT:
A new method is described for the preparation of a safe, immunogenic and efficacious vaccine for protection against the disease pertussis. In development of this vaccine, specific functional sites of **pertussis toxin** have been identified, and using this information, defined mutant holotoxins have been produced by site directed mutagenesis of the toxin gene. A number of these holotoxin analogues are detoxified, retain an immunodominant S1 epitope, are immunogenic and are protective in the standard pertussis vaccine potency test in mice.

US PAT NO: 5,332,583 [IMAGE AVAILABLE] L2: 6 of 9
TITLE: Vaccine containing genetically-detoxified pertussis holotoxin
DATE ISSUED: Jul. 26, 1994
INVENTOR: Michel H. Klein, Willowdale, Canada
Heather A. Boux, Aurora, Canada
Stephen A. Cockle, Richmond Hill, Canada
Sheena M. Loosmore, Aurora, Canada
Gavin R. Zealey, Concord, Canada
SEARCH-FLD: 435/69.7, 252.3, 68.1, 193, 194, 252.4, 253.6, 252.1, 71.2, 243, 248, 832; 424/92, 88; 530/403-406, 350, 387

ABSTRACT:
A new method is described for the preparation of a safe, immunogenic and efficacious vaccine for protection against the disease pertussis. In development of this vaccine, specific functional sites of **pertussis toxin** have been identified, and using this information, defined mutant holotoxins have been produced by site directed mutagenesis of the toxin gene. A number of these holotoxin analogues are detoxified, retain an immunodominant S1 epitope, are immunogenic and are protective in the standard pertussis vaccine potency test in mice.

US PAT NO: 5,244,657 [IMAGE AVAILABLE] L2: 7 of 9
TITLE: Genetic detoxification of **pertussis toxin**
DATE ISSUED: Sep. 14, 1993
INVENTOR: Michel H. Klein, Willowdale, Canada

Heather A. Boux, Aurora, Canada
Stephen A. Cockle, Richmond Hill, Canada
Sheena M. Loosmore, Aurora, Canada
Gavin R. Zealey, Concord, Canada
SEARCH-FLD: 424/88, 92

ABSTRACT:
A new method is described for the preparation of a safe, immunogenic and efficacious vaccine for protection against the disease pertussis. In development of this vaccine, specific functional sites of **pertussis toxin** have been identified, and using this information, defined mutant holotoxins have been produced by site directed mutagenesis of the toxin gene. A number of these holotoxin analogs are detoxified, retain an immunodominant S1 epitope, are immunogenic and are protective in the standard pertussis vaccine potency test in mice.

US PAT NO: 5,221,618 [IMAGE AVAILABLE] L2: 8 of 9
TITLE: Genetic detoxification of **pertussis toxin**
DATE ISSUED: Jun. 22, 1993
INVENTOR: Michel H. Klein, Willowdale, Canada
Heather A. Boux, Aurora, Canada
Stephen A. Cockle, Richmond Hill, Canada
Sheena M. Loosmore, Aurora, Canada
Gavin R. Zealey, Concord, Canada
SEARCH-FLD: 435/69.1, 69.3, 252.1, 172.3; 536/27; 935/10, 11, 12, 65

ABSTRACT:
A new method is described for the preparation of a safe, immunogenic and efficacious vaccine for protection against the disease pertussis. In development of this vaccine, specific functional sites of **pertussis toxin** have been identified, and using this information, defined mutant holotoxins have been produced by site directed mutagenesis of the toxin gene. A number of these toxin analogues are detoxified, retain an immunodominant S1 epitope, are immunogenic and are protective in the standard pertussis vaccine potency test in mice.

US PAT NO: 5,085,862 [IMAGE AVAILABLE] L2: 9 of 9
TITLE: Genetic detoxification of **pertussis toxin**
DATE ISSUED: Feb. 4, 1992
INVENTOR: Michel H. Klein, Willowdale, Canada
Heather A. Boux, Aurora, Canada
Stephen A. Cockle, Richmond Hill, Canada
Sheena M. Loosmore, Aurora, Canada
Gavin R. Zealey, Concord, Canada
SEARCH-FLD: 424/92; 435/252.3; 530/350, 387, 403, 405, 406

ABSTRACT:
A new method is described for the preparation of a safe, immunogenic and efficacious vaccine for protection against the disease pertussis. In development of this vaccine, specific functional sites of **pertussis toxin** have been identified, and using this information, defined

mutant holotoxins have been produced by site directed mutagenesis of the toxin gene. A number of these toxin analogues are detoxified, retain an immunodominant S1 epitope, are immunoganic and are protective in the standard pertussis vaccine potency test in mice.

=> d his l3-; d 1-5 .bevpat

(FILE 'USPAT' ENTERED AT 15:31:41 ON 27 JUL 1999)
L3 1 S BARCHFELD, G?/IN
L4 0 S (DELGIUDICE, G? OR DEL GIUDICE, G?)/IN
L5 10 S RAPPUOLI, R?/IN
L6 0 S L3 AND L5
L7 5 S (L3 OR L5) AND L1
L8 5 S L7 NOT L2

- Author(s)

US PAT NO: 5,925,546 [IMAGE AVAILABLE] L8: 1 of 5
TITLE: Immunologically active polypeptides with altered toxicity useful for the preparation of an antipertussis vaccine
DATE ISSUED: Jul. 20, 1999
INVENTOR: Mariagrazia Pizza, Siena, Italy
Antonella Bartoloni, Siena, Italy
Rino Rappuoli, Siena, Italy
SEARCH-FLD: 530/350; 424/240.1, 190.1, 254.1, 832; 435/69.1, 69.3, 172.3, 320.1; 536/23.7

ABSTRACT:
Immunological active polypeptides with no or reduced toxicity useful for the preparation of an antipertussis vaccine. Method for the preparation of said polypeptides which comprises, cultivating a microorganism transformed with a hybrid plasmid including the gene/s which codes for at least one of said polypeptides in a suitable medium and recovering the desired polypeptide from the cells or from the culture medium.

US PAT NO: 5,908,825 [IMAGE AVAILABLE] L8: 2 of 5
TITLE: Dosage composition for nasal delivery and method of use of the same
DATE ISSUED: Jun. 1, 1999
INVENTOR: Alessio Fasano, Ellicott City, MD
Teresa De Magistris, Siena, Italy
Sergio Uzzau, Sassari, Italy
Rino Rappuoli, Querciegrossa, Italy
SEARCH-FLD: 514/2, 3, 12, 15, 4, 866; 424/261.1, 130.1, 184.1; 530/303, 362, 351, 387.1, 399

ABSTRACT:
A nasal dosage composition for nasal delivery comprising (A) a therapeutic agent; and (B) zonula occludens toxin, as well as a method for the use of the same.

US PAT NO: 5,889,172 [IMAGE AVAILABLE] L8: 3 of 5
TITLE: DNA sequences for immunologically active peptides of **pertussis toxin**
DATE ISSUED: Mar. 30, 1999
INVENTOR: Mariagrazia Pizza, Siena, Italy
Antonella Bartoloni, Siena, Italy
Rino Rappuoli, Siena, Italy
SEARCH-FLD: 536/23.7; 435/320.1, 69.1, 69.3, 172.3; 424/190.1, 254.1, 833

ABSTRACT:
Immunologically active polypeptides with no or reduced toxicity useful for the preparation of an antipertussis vaccine. Method for the preparation of said polypeptides which comprises, cultivating a microorganism transformed with a hybrid plasmid including the gene/s which codes for at least one of said polypeptides in a suitable medium and recovering the desired polypeptide from the cells or from the culture medium.

US PAT NO: 5,785,971 [IMAGE AVAILABLE] L8: 4 of 5
TITLE: **Pertussis toxin** and use in vaccines
DATE ISSUED: Jul. 28, 1998
INVENTOR: **Rino Rappuoli**, Quercegrossa-Monteriggioni, Italy
Alfredo Nicosia, Siena, Italy
Maria Beatrice Arico, Quercegrossa, Italy
SEARCH-FLD: 530/350; 514/12; 424/190.1, 240.1, 254.1; 435/69.1, 193

ABSTRACT:
Cloning and sequencing of the Eco RI fragment of B. pertussis chromosomal DNA with 4696 base pairs, containing the genes which code for the five subunits of the **pertussis toxin**.
A hybrid plasmid containing the DNA fragment or its further fragments and a micro-organism transformed by the hybrid plasmid and capable of expressing the cloned DNA fragment or further fragments thereof by synthesis of the **pertussis toxin** or one or more subunits of the **pertussis toxin**.
The **pertussis toxin** or one or more subunits of the **pertussis toxin** so obtained are useful for the preparation of vaccines and diagnostic kits.

US PAT NO: 5,427,788 [IMAGE AVAILABLE] L8: 5 of 5
TITLE: **Pertussis toxin** and use in vaccines
DATE ISSUED: Jun. 27, 1995
INVENTOR: **Rino Rappuoli**, Quercegrossa-Monteriggioni, Italy
Alfredo Nicosia, Siena, Italy
Maria B. Arico', Quercegrossa, Italy
SEARCH-FLD: 514/12; 435/193, 69.1; 424/190.1, 240.1, 254.1

ABSTRACT:
Cloning and sequencing of the Eco RI fragment of B. pertussis chromosomal DNA with 4696 base pairs, containing the genes which code for the five

subunits of the **pertussis toxin**.
A hybrid plasmid containing the DNA fragment or its further fragments and a micro-organism transformed by the hybrid plasmid and capable of expressing the cloned DNA fragment or further fragments thereof by synthesis of the **pertussis toxin** or one or more subunits of the **pertussis toxin**.
The **pertussis toxin** or one or more subunits of the **pertussis toxin** so obtained are useful for the preparation of vaccines and diagnostic kits.

=> fil hom

FILE 'HOME' ENTERED AT 15:34:51 ON 27 JUL 1999

Devi, S.
09/044696


```
       27jul99 14:34:29 User219783 Session D1483.2
SYSTEM:OS  - DIALOG OneSearch
  File  65:Inside Conferences  1993-1999/June W2
        (c) 1999 BLDSC all rts. reserv.
  File  35:Dissertation Abstracts Online  1861-1999/Jul
        (c) 1999 UMI
  File  77:Conference Papers Index  1973-1999/Jul
        (c) 1999 Cambridge Sci Abs
  File 144:PASCAL  1973-1999/JUN
        (c) 1999 INIST/CNRS
  File 266:FEDRIP  1999/May
        Comp & dist by NTIS, Intl Copyright All Rights Res

      Set  Items  Description
      ---  -----  -----------
? ds

Set     Items   Description
S1       7644   (ADP(W)RIBOSYLAT? OR CHOLER? OR PERTUSS? OR COLI)(3W)TOXIN?
             ? OR (CT OR PT OR LT)(10N)TOXIN? ?
S2          4   S1 AND ((DETOX? OR DE(W)(TOXIF? OR TOX????))(5N)(MUTANT? ?
            OR MUTAGEN? OR MUTAT?))
S3          4   RD (unique items)
? t 3/3,ab/1-4

>>>No matching display code(s) found in file(s): 65

 3/3,AB/1     (Item 1 from file: 144)
DIALOG(R)File 144:PASCAL
(c) 1999 INIST/CNRS. All rts. reserv.

  13712203   PASCAL No.: 98-0403290
  Mucosal immunogenicity of genetically detoxified derivatives of heat
labile toxin from Escherichia coli
  DOUCE G; GIULIANI M M; GIANNELLI V; PIZZA M G; RAPPUOLI R; DOUGAN G
  Department of Biochemistry, Imperial College of Science, Technology and
Medicine, Exhibition Road, London SW7 2AY, United Kingdom; The Chiron
Vaccines Immunological Research Institute, Via Fiorentina 1, Siena 53100,
Italy
  Journal: Vaccine,  1998, 16 (11-12) 1065-1073
  Language: English
  Using  a  fixed  dose of antigen, the immune response to detoxified
mutants of LT-WT following intranasal (i.n.), subcutaneous (s.c.) and
oral  (i.g.)  immunisation has been studied. When given i.n., both LT
-WT  and  mutant  toxin, K63, generated significant levels of
toxin -specific  IgG in the serum, and the levels of IgA in nasal and
lung  lavages were greater than those induced by rLT-B. In comparison, i.g.
immunisation  of  mice with a similar quantity of either LT-WT or K63
toxin  induced  barely detectable levels of IgG in the sera. However,
```

- key terms

if the amount of protein used for i.g. immunisation was increased tenfold, relatively good levels of **toxin**-specific IgG were induced in the sera by both **LT**-WT or K63. Low levels of **toxin**-specific IgA were also observed in intestinal washes from these mice. Western blotting of the sera, using the native toxin as an antigen, demonstrated the presence of both anti-A and anti-B subunit antibodies, Most significantly, toxin-neutralising antibodies were induced in the serum, with the strongest activity being induced by the LT-WT, an intermediate activity induced by mutant K63 and a lower response by rLT-B. Together, these data show that ADP-ribosyltransferase is not necessary for mucosal immunogenicity of these proteins, and that the i.n. route of immunisation is more effective than the i.g. route of immunisation for the generation of both systemic (IgG) and mucosal (IgA) immune responses.

3/3,AB/2 (Item 2 from file: 144)
DIALOG(R)File 144:PASCAL

11785230 PASCAL No.: 94-0662718
Etude de quatre facteurs de virulence de Bordetella pertussis dans un modele murin d'infection respiratoire
(Study of four virulence factors of Bordetella pertussis by using a murine model of respiratory infection)
KHELEF Nadia; GUISO Nicole, dir
Universite de Paris 11, Francee
Univ.: Universite de Paris 11. FRA Degree: Th. doct.
1994-06; 1994 240 p.
Language: French Summary Language: French; English

Bordetella pertussis, l'agent de la coqueluche, synthetise plusieurs facteurs de virulence, parmi lesquels deux adhesines, la pertactine et l'hemagglutinine filamenteuse, et deux toxines, la toxine de pertussis et l'adenylcyclase-hemolysine. Leur role a ete etudie a l'aide de mutants n'exprimant pas l'un de ces facteurs dans un modele murin d'infection respiratoire, au niveau de la colonisation et des lesions pulmonaires. Nous avons montre que chacune des adhesines pouvait compenser l'absence de l'autre, mais qu'elles avaient un role dans la persistance bacterienne, suggerant l'existence d'une cooperation entre ces adhesines. A l'inverse, l'adenylcyclase-hemolysine est indispensable a la multiplication bacterienne initiale, alors que la toxine de pertussis est importante en fin d'infection. Les mutants depourvus d'une des toxines induisent peu ou pas d'inflammation pulmonaire, indiquant que ces toxines participeraient a cet effet, soit directement, soit indirectement, en inactivant les cellules immunitaires et en facilitant ainsi l'action d'autres facteurs proinflammatoires. L'etude des interactions cellulaires in vitro a revele que B. pertussis etait cytotoxique pour les macrophages murins qui meurent par apoptose ou mort cellulaire programmee. Le **mutant** depourvu

de toxine de pertussis est aussi actif que la souche sauvage, alors que celui qui n'exprime pas d'adenylcyclase-hemolysine n'est plus toxique, suggerant un role pour l'adenylcyclase-hemolysine dans l'apoptose des macrophages. Enfin, nous avons mis en evidence que, malgre leur efficacite contre une infection respiratoire murine par B. pertussis, aucun des quatre facteurs synthetises par B. pertussis ne protegeait contre une infection par Bordetella parapertussis, l'autre espece pathogene pour l'homme, suggerant que cette protection serait specifique d'espece

3/3,AB/3 (Item 3 from file: 144)
DIALOG(R)File 144:PASCAL

10652240 PASCAL No.: 93-0161524
Progress towards the development of new vaccines against whooping cough
RAPPUOLI R; PODDA A; PIZZA M; COVACCI A; BARTOLONI A; DE MAGISTRIS M T; NENCIONI L
Immunobiology resz. inst. Siena, 53100 Siena, Italy
Journal: Vaccine, 1992, 10 (14) 1027-1032
Language: English
Acellular vaccines against whooping cough are in the final stage of clinical testing and are likely to become available for mass immunization in the near future. Over a dozen vaccines of similar composition have been developed by vaccine companies and research laboratories; all of them contain a detoxified form of **pertussis toxin** (**PT**) that may be present alone or combined with one or more other non-toxic proteins, such as filamentous haemagglutinin (FHA), pertactin (69 kDa), and the agglutinogens (AGG)

3/3,AB/4 (Item 4 from file: 144)
DIALOG(R)File 144:PASCAL

08820365 PASCAL No.: 89-0369737
BREVET. Genetic detoxification of **pertussis toxin**
CONNAUGHT LABORATORIES LTD
Publication Date: 1989-06-28
Patent: EP 0322115 A2 Patent Filing: 88311133.8, 1988-11-24
Convention: GB 8727489, 1987-11-24 IPC: C 12N 15/00
Language: English

Set	Items	Description
S4	11	AU=(BARCHFELD, G? OR BARCHFELD G?)
S5	27	AU=(DELGIUDICE, G? OR DELGIUDICE G? OR DEL GUIDICE, G? OR - DEL GUIDICE G?)
S6	207	AU=(RAPPUOLI, R? OR RAPPUOLI R?)
S7	0	S4 AND S5 AND S6

– Author (s)

Searcher : Shears 308-4994

S8 1 S4 AND (S6 OR S5)
S9 0 S5 AND S6
S10 1 S8 NOT S2
? t 10/3,ab/1

>>>No matching display code(s) found in file(s): 65

10/3,AB/1 (Item 1 from file: 144)
DIALOG(R)File 144:PASCAL

14003751 PASCAL No.: 99-0188851
The adjuvants MF59 and LT-K63 enhance the mucosal and systemic immunogenicity of subunit influenza vaccine administered intranasally in mice
BARCHFELD G L; HESSLER A L; CHEN M; PIZZA M; **RAPPUOLI R**; VAN NEST G A
Chiron Corporation, 4560 Horton Street, Emeryville, CA 94608-2916, United States; Chiron Vaccines, Via Fiorentina 1, 53100 Siena, Italy
Journal: Vaccine, 1999, 17 (7-8) 695-704
Language: English
Commercial influenza vaccines generate serum antibody, but not local IgA. Influenza vaccines that induce both serum and secretory antibody are more likely to protect against infection and disease progression. The adjuvants MF59 and LT-K63 were tested intramuscularly and intranasally with subunit HA. In naive mice, intranasal adjuvant effect was more apparent when included with the first than second immunization. In previously infected mice, intranasal adjuvants had little effect on serum antibodies and were most effective for nasal antibodies after the second immunization. Overall, both adjuvants enhanced anti-HA IgA and IgG by intranasal vaccination whereas, by intramuscular vaccination, they only enhanced serum IgG.

? log y

27jul99 14:42:22 User219783 Session D1483.3

Devi, S.
09/044696

(FILE 'CAPLUS' ENTERED AT 15:14:03 ON 27 JUL 1999)

-key terms

=> d que

L1 15389 SEA FILE=CAPLUS ABB=ON PLU=ON (ADP(W)RIBOSYLAT? OR CHOLER? OR PERTUSS? OR COLI)(3W)TOXIN OR (CT OR PT OR LT)(S)TOXIN
L3 15 SEA FILE=CAPLUS ABB=ON PLU=ON L1 AND (DETOX? OR DE TOX?)(5A)(MUTANT OR MUTAGEN? OR MUTAT?)

=> d 1-15 .bevstr1

L3 ANSWER 1 OF 15 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1998:672490 CAPLUS
DOCUMENT NUMBER: 129:289177
TITLE: **Detoxified mutants of** bacterial **ADP-ribosylating toxins** as parenteral adjuvants
INVENTOR(S): Barchfeld, Gail; Del Giudice, Giuseppe; Rappuoli, Rino
PATENT ASSIGNEE(S): Chiron Corporation, USA
SOURCE: PCT Int. Appl., 51 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9842375	A1	19981001	WO 98-US5454	19980319
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9865713	A1	19981020	AU 98-65713	19980319
PRIORITY APPLN. INFO.:			US 97-41227	19970321
			US 98-44696	19980318
			WO 98-US5454	19980319

AB The present invention provides parenteral adjuvants comprising **detoxified mutants** of bacterial **ADP-ribosylating toxins**, esp. **pertussis toxin (PT)**, **cholera toxin (CT)**, and Escherichia **coli**-derived heat-labile

toxin (LT). The immune adjuvant includes LT-K63, LT-R72, CT-S109 and PT-K9/G129. LT-K63 was prepd. as parenteral adjuvant for vaccine comprising herpes simplex virus type 2 gD antigen, influenza hemagglutinin, and HIV p24 gag.

IT Diphtheria toxin
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(CRM197; **detoxified mutants** of bacterial **ADP-ribosylating toxins** as parenteral adjuvants)

IT Heat labile enterotoxin
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(Escherichia coli; **detoxified mutants** of bacterial **ADP-ribosylating toxins** as parenteral adjuvants)

IT Toxins
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(bacterial ADP-ribosylating; **detoxified mutants** of bacterial **ADP-ribosylating toxins** as parenteral adjuvants)

IT Adjuvants (immunological)
Bacteria (Eubacteria)
Escherichia coli
Human herpesvirus 2
Human immunodeficiency virus
Influenza
Intramuscular injections
Parenteral solutions (drug delivery systems)
Protein sequences
Subcutaneous injections
Topical drug delivery systems
Vaccines
Vibrio cholerae
(**detoxified mutants** of bacterial **ADP-ribosylating toxins** as parenteral adjuvants)

IT Antigens
Cholera toxin
Glycoprotein D
Hemagglutinins
Pertussis toxin
p24 (gag protein)
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(**detoxified mutants** of bacterial **ADP-ribosylating toxins** as parenteral adjuvants)

IT Drug delivery systems
(transcutaneous; **detoxified mutants** of bacterial **ADP-ribosylating toxins** as parenteral adjuvants)

IT Vertebrate (Vertebrata)
(vaccine; **detoxified mutants** of bacterial

ADP-ribosylating toxins as parenteral adjuvants)

IT 214068-46-9 214068-50-5 214068-55-0
RL: PRP (Properties)
(amino acid sequence; **detoxified mutants** of bacterial **ADP-ribosylating toxins** as parenteral adjuvants)

L3 ANSWER 2 OF 15 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1998:476941 CAPLUS
DOCUMENT NUMBER: 129:243790
TITLE: Mucosal immunogenicity of genetically detoxified derivatives of heat labile toxin from Escherichia coli
AUTHOR(S): Douce, Gill; Giuliani, Marzia Monica; Giannelli, Valentina; Pizza, Maria Grazia; Rappuoli, Rino; Dougan, Gordon
CORPORATE SOURCE: Department of Biochemistry, Imperial College of Science, Technology and Medicine, London, SW7 2AY, UK
SOURCE: Vaccine (1998), 16(11/12), 1065-1073
CODEN: VACCDE; ISSN: 0264-410X
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Using a fixed dose of antigen, the immune response to **detoxified mutants** of LT-WT following intranasal (i.n.), s.c. and oral (i.g.) immunization has been studied. When given i.n., both **LT**-WT and mutant **toxin**, K63, generated significant levels of **toxin**-specific IgG in the serum, and the levels of IgA in nasal and lung lavages were greater than those induced by rLT-B. In comparison, i.g. immunization of mice with a similar quantity of either **LT**-WT or K63 **toxin** induced barely detectable levels of IgG in the sera. However, if the amt. of protein used for i.g. immunization was increased tenfold, relatively good levels of **toxin** -specific IgG were induced in the sera by both **LT**-WT or K63. Low levels of toxin-specific IgA were also obsd. in intestinal washes from these mice. Western blotting of the sera, using the native toxin as an antigen, demonstrated the presence of both anti-A and anti-B subunit antibodies. Most significantly, **toxin** -neutralizing antibodies were induced in the serum, with the strongest activity being induced by the **LT**-WT, an intermediate activity induced by mutant K63 and a lower response by rLT-B. Together, these data show that ADP-ribosyltransferase is not necessary for mucosal immunogenicity of these proteins, and that the i.n. route of immunization is more effective than the i.g. route of immunization for the generation of both systemic (IgG) and mucosal (IgA) immune responses.

IT Toxins
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(heat labile; mucosal immunogenicity of genetically detoxified derivs. of heat labile toxin from Escherichia coli)
IT Vaccination
(intranasal; mucosal immunogenicity of genetically detoxified derivs. of heat labile toxin from Escherichia coli)
IT Lung
(lavage; mucosal immunogenicity of genetically detoxified derivs. of heat labile toxin from Escherichia coli)
IT Escherichia coli
Mucosal immunity
Nasal mucosa
Serum (blood)
(mucosal immunogenicity of genetically detoxified derivs. of heat labile toxin from Escherichia coli)
IT IgA
IgG
RL: MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative)
(mucosal immunogenicity of genetically detoxified derivs. of heat labile toxin from Escherichia coli)
IT Immunization
(oral; mucosal immunogenicity of genetically detoxified derivs. of heat labile toxin from Escherichia coli)

L3 ANSWER 3 OF 15 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1998:337192 CAPLUS
DOCUMENT NUMBER: 129:107991
TITLE: **Pertussis toxin** potentiates Th1 and Th2 responses to co-injected antigen: adjuvant action is associated with enhanced regulatory cytokine production and expression of the co-stimulatory molecules B7-1, B7-2 and CD28
AUTHOR(S): Ryan, Mark; Mccarthy, Leone; Rappuoli, Rino; Mahon, Bernard P.; Mills, Kingston H. G.
CORPORATE SOURCE: Infection and Immunity Group, Department of Biology, National University of Ireland, Kildare, Ire.
SOURCE: Int. Immunol. (1998), 10(5), 651-662
CODEN: INIMEN; ISSN: 0953-8178
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **Pertussis toxin** (**PT**) is a major virulence factor of Bordetella pertussis which exerts a range of effects on the immune system, including the enhancement of IgE, IgA and IgG prodn., delayed-type hypersensitivity reactions, and the

induction of exptl. autoimmune diseases. However, the mechanism by which PT mediates adjuvanticity remains to be defined. In this investigation the authors have shown that PT can potentiate antigen-specific T cell proliferation and the secretion of IFN-.gamma., IL-2, IL-4 and IL-5 when injected with foreign antigens. A chem. **detoxified** PT and a genetic **mutant** with substitutions/deletions in the S-1 and B oligomer components that abrogate enzymic and binding activity displayed no adjuvant properties. In contrast, a non-toxic S-1 mutant devoid of enzymic activity but still capable of receptor binding retained its adjuvanticity, augmenting the activation of both Th1 and Th2 subpopulations of T cells. To address the mechanism of T cell activation, the authors found that PT stimulated the prodn. of IFN- and IL-2 by naive T cells and IL-1 by macrophages. Therefore potentiation of distinct T cell subpopulations may have resulted in part from the pos. influence of IFN-.gamma. on the development of Th1 cells and the co-stimulatory role of IL-1 for Th2 cells. Furthermore, PT augmented expression of the co-stimulatory mols. B7-1 and B7-2 on macrophages and B cells, and CD28 on T cells, suggesting that the adjuvant effect may also be assocd. with facilitation of the second signal required for maximal T cell activation. This study demonstrates that the immunopotentiating properties of PT are largely independent of ADP-ribosyltransferase activity, but are dependent on receptor binding activity and appear to involve enhanced activation of T cells.

IT Th1 cell
Th2 cell
(adjuvant activity of **pertussis toxin** for)

IT B cell (lymphocyte)
Macrophage
(adjuvant activity of **pertussis toxin** in relation to induced costimulatory mol. expression by accessory cell)

IT CD28 (antigen)
Interferon .gamma.
Interleukin 2
Interleukin 4
Interleukin 5
RL: MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative)
(adjuvant activity of **pertussis toxin** in relation to induced expression by T-cell for)

IT CD80 (antigen)
CD86 (antigen)
Interleukin 1
RL: MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative)
(adjuvant activity of **pertussis toxin** in

relation to induced expression by accessory cell for)
IT Immunostimulation
(cellular; costimulatory mol. and regulatory cytokine expression in adjuvant activity of **pertussis toxin**)
IT T cell activation
(costimulatory mol. and regulatory cytokine expression in adjuvant activity of **pertussis toxin**)
IT **Pertussis toxin**
RL: BAC (Biological activity or effector, except adverse); PRP (Properties); BIOL (Biological study)
(mechanism of adjuvant activity of)

L3 ANSWER 4 OF 15 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1998:293623 CAPLUS
DOCUMENT NUMBER: 128:279567
TITLE: Immunogenic **detoxified mutant** Escherichia **coli LT**-A **toxin**
INVENTOR(S): Pizza, Mariagrazia; Giuliani, Marzia Monica; Rappuoli, Rino
PATENT ASSIGNEE(S): Chiron S.P.A., Italy; Pizza, Mariagrazia; Giuliani, Marzia Monica; Rappuoli, Rino
SOURCE: PCT Int. Appl., 69 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9818928	A1	19980507	WO 97-IB1440	19971030
W: CA, JP, US				
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
PRIORITY APPLN. INFO.:			GB 96-22660	19961031

AB An immunogenic detoxified protein is provided which comprises the amino acid sequence of subunit A of an E. **coli** heat labile **toxin** (**LT**-A) or a fragment thereof in which at least amino acid Ala-72 of the A subunit is mutated, preferably by substitution with Arg. The toxoid is useful as vaccine against an enterotoxigenic strain of E. coli and is produced by recombinant DNA means by site-directed mutagenesis. A 1.5 kb SmaI-EcoRI fragment from plasmid pEWD299 contg. the gene for LT-A and the LT promoter region was subcloned to produce vector BS-LT-A. BS-LT-A was mutagenized with oligonucleotide oligoLT-A72R to change the Ala-72 codon to the Arg codon and ligated to the EcoRI-HindIII fragment contg. the gene for LT-B and cloned to produce vector BS-LTA72R. E. coli was transformed with BS-LTA72R and the LT-A72R mutant purified.

ADP-ribosylation of LT-A72R was lower than wild type LT-A, the toxicity of LT-A72R was 10-5 lower than wild type LT-A, and LT-A72R proved to be an effective mucosal adjuvant.

IT Adjuvants (immunological)
Detoxification (metabolic)
Escherichia coli
Genetic engineering
Site directed **mutagenesis**
Vaccines
(immunogenic **detoxified mutant** Escherichia **coli LT-A toxin**)

IT Antigens
Heat labile enterotoxin
RL: BPN (Biosynthetic preparation); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(immunogenic **detoxified mutant** Escherichia **coli LT-A toxin**)

IT 56-41-7DP, Alanine, residue-72
RL: BPN (Biosynthetic preparation); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(immunogenic **detoxified mutant** Escherichia **coli LT-A toxin** with Ala-72 mutated by substitution)

IT 74-79-3DP, Arginine, residue-72
RL: BPN (Biosynthetic preparation); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(immunogenic **detoxified mutant** Escherichia **coli LT-A toxin** with Ala-72 mutated by substitution with Arg)

L3 ANSWER 5 OF 15 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1998:198478 CAPLUS
DOCUMENT NUMBER: 128:307201
TITLE: Recent advances in immunological adjuvants: the development of particulate antigen delivery systems
AUTHOR(S): O'hagan, Derek T.
CORPORATE SOURCE: Chiron Corporation, Emeryville, CA, 94704, USA
SOURCE: Expert Opin. Invest. Drugs (1998), 7(3), 349-359
CODEN: EOIDER; ISSN: 1354-3784
PUBLISHER: Ashley Publications
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English

AB A review with 70 refs. New generation vaccines, including those based on recombinant proteins, are safer than traditional vaccines, but are less immunogenic. Therefore, there is an urgent need for

the development of new and improved vaccine adjuvants. A no. of potent immunostimulatory mols. obtained from bacterial cells or plants have been extensively evaluated as adjuvants. However, a no. of these mols. have displayed significant toxicity, both in preclin. animal models and in human clin. trials. An alternative approach to the development of novel adjuvants involves the prepn. of particulate antigen delivery systems of similar dimensions to natural pathogens. In the absence of addnl. immunostimulatory mols., emulsion droplets and microparticles have been shown to be potent adjuvants for the induction of both humoral and cell-mediated immune responses following systemic administration. Moreover, particulate delivery systems have been shown to display an acceptable toxicity profile in a no. of clin. trials. Particulate antigen delivery systems also have the potential to function as potent adjuvants following administration by mucosal routes, including oral and intranasal. An alternative approach to the mucosal delivery of vaccines involves the use of genetically **detoxified mutant toxins**, e.g., **LT**-K63, as mucosal adjuvants. The use of novel adjuvants and antigen delivery systems is likely to extend the use of vaccines into the area of therapeutics, involving the eradication of infectious diseases and cancers, or the amelioration of autoimmune disorders.

IT Adjuvants (immunological)
Drug delivery systems
Vaccines
(recent advances in immunol. adjuvants and the development of particulate antigen delivery systems)

IT Antigens
RL: BAC (Biological activity or effector, except adverse); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(recent advances in immunol. adjuvants and the development of particulate antigen delivery systems)

L3 ANSWER 6 OF 15 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1997:557657 CAPLUS
DOCUMENT NUMBER: 127:219543
TITLE: Immunogenic **detoxified mutants** of **cholera toxin**
INVENTOR(S): Fontana, Maria Rita; Pizza, Mariagrazia; Rappuoli, Rino
PATENT ASSIGNEE(S): Chiron S.P.A., Italy; Fontana, Maria Rita; Pizza, Mariagrazia; Rappuoli, Rino
SOURCE: PCT Int. Appl., 53 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9729771	A1	19970821	WO 97-IB183	19970217
W: CA, JP, US				
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
CA 2244800	AA	19970821	CA 97-2244800	19970217
EP 880361	A1	19981202	EP 97-902552	19970217
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
PRIORITY APPLN. INFO.:			GB 96-3314	19960216
			WO 97-IB183	19970217

AB An immunogenic detoxified protein comprising the amino acid sequence of subunit A of a **cholera toxin** or a fragment thereof in which at least one amino acid is substituted with another amino acid characterized in that, in purified form, the immunogenic detoxified protein exhibits a residual toxicity greater than 10000 fold lower than its naturally occurring counterpart. In the described embodiment, the amino acid at, or in a position corresponding to Pro-106 is replaced with another amino acid. The immunogenic detoxified protein is useful as vaccine for Vibrio cholerae and is produced by recombinant DNA means by site-directed mutagenesis. Vaccine comprises the detoxified **cholera toxin** and a second antigen are also disclosed.

IT **Cholera toxin**
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(**detoxified**; immunogenic **detoxified mutants** of **cholera toxin**)

IT DNA
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(encoding **detoxified cholera toxin**; immunogenic **detoxified mutants** of **cholera toxin**)

IT Vaccines
Vibrio cholerae
(immunogenic **detoxified mutants** of **cholera toxin**)

IT Antigens
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(immunogenic **detoxified mutants** of **cholera toxin**)

L3 ANSWER 7 OF 15 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1995:801623 CAPLUS
DOCUMENT NUMBER: 123:196584
TITLE: Non-toxic mucosal adjuvant
INVENTOR(S): Rappuoli, Rino
PATENT ASSIGNEE(S): Biocine S.p.A., Italy

SOURCE: PCT Int. Appl., 29 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9517211	A1	19950629	WO 95-IB13	19941222
W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ				
RW: KE, MW, SD, SZ, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
CA 2179771	AA	19950629	CA 94-2179771	19941222
AU 9512785	A1	19950710	AU 95-12785	19941222
EP 732937	A1	19960925	EP 95-903889	19941222
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				

PRIORITY APPLN. INFO.: GB 93-26174 19931222
WO 94-IB68 19940324
WO 95-IB13 19941222

AB A non-toxic mucosal adjuvant is provided which may be admixed with further antigens to provide a vaccine administrable to mucosal surfaces in organisms including man. Preferably, the non-toxic mucosal adjuvant is a **detoxified mutant** of a bacterial **ADP-ribosylating toxin**, optionally comprising one or more amino aid addns., deletions or substitutions. The non-toxic mucosal adjuvant may also be a **detoxified mutant** of **cholera toxin** or heat-labile toxin.

IT Toxins
RL: MOA (Modifier or additive use); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(ADP-ribosylating; bacterial ADP-ribosylating or **cholera** or heat-labile **toxins** as nontoxic mucosal adjuvant for antigen vaccine)

IT Bacteria
Mucous membrane
Vaccines
(bacterial ADP-ribosylating or **cholera** or heat-labile **toxins** as nontoxic mucosal adjuvant for antigen vaccine)

IT Antigens
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(bacterial ADP-ribosylating or **cholera** or heat-labile **toxins** as nontoxic mucosal adjuvant for antigen vaccine)

IT Toxins
RL: MOA (Modifier or additive use); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(holo-; bacterial ADP-ribosylating or **cholera** or heat-labile **toxins** as nontoxic mucosal adjuvant for antigen vaccine)

IT Immunostimulants
(adjuvants, bacterial ADP-ribosylating or **cholera** or heat-labile **toxins** as nontoxic mucosal adjuvant for antigen vaccine)

IT Toxins
RL: MOA (Modifier or additive use); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(cholera, bacterial ADP-ribosylating or **cholera** or heat-labile **toxins** as nontoxic mucosal adjuvant for antigen vaccine)

IT Toxins
RL: MOA (Modifier or additive use); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(heat-labile, bacterial ADP-ribosylating or **cholera** or heat-labile **toxins** as nontoxic mucosal adjuvant for antigen vaccine)

IT Pharmaceutical dosage forms
(nasal, bacterial ADP-ribosylating or **cholera** or heat-labile **toxins** as nontoxic mucosal adjuvant for antigen vaccine)

IT Pharmaceutical dosage forms
(oral, bacterial ADP-ribosylating or **cholera** or heat-labile **toxins** as nontoxic mucosal adjuvant for antigen vaccine)

L3 ANSWER 8 OF 15 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1994:653248 CAPLUS
DOCUMENT NUMBER: 121:253248
TITLE: A genetically detoxified derivative of heat-labile Escherichia coli enterotoxin induces neutralizing antibodies against the A subunit
AUTHOR(S): Pizza, Mariagrazia; Fontana, Maria Rita; Giuliani, Marzia M.; Domenighini, Mario; Magagnoli, Claudia; Giannelli, Valentina; Nucci, Daniele; Hol, Wim; Manetti, Roberto; Rappuoli, Rino
CORPORATE SOURCE: Immunobiological Res. Inst. Siena, Siena, 53100, Italy
SOURCE: J. Exp. Med. (1994), 180(6), 2147-54
CODEN: JEMEAV; ISSN: 0022-1007
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Escherichia coli enterotoxin (**LT**) and the homologous

cholera toxin (**CT**) are A-B **toxins** that cause travelers' diarrhea and cholera, resp. So far, exptl. live and killed vaccines against these diseases have been developed using only the nontoxic B portion of these toxins. The enzymically active A subunit has not been used because it is responsible for the toxicity and it is reported to induce a negligible titer of toxin neutralizing antibodies. Site-directed mutagenesis was used to inactivate the ADP-ribosyltransferase activity of the A subunit. Nontoxic derivs. of LT were obtained that elicited a good titer of neutralizing antibodies recognizing the A subunit.

IT Escherichia coli
Protein sequences
(Escherichia coli detoxified enterotoxin deriv. induces neutralizing antibodies against A subunit)

IT Vaccines
(for enterotoxigenic Escherichia coli; Escherichia coli detoxified enterotoxin deriv. induces neutralizing antibodies against A subunit)

IT Toxins
RL: BAC (Biological activity or effector, except adverse); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(entero-, A subunit; Escherichia coli detoxified enterotoxin deriv. induces neutralizing antibodies against A subunit)

IT Antibodies
RL: BPR (Biological process); MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative); PROC (Process)
(neutralizing, Escherichia coli detoxified enterotoxin deriv. induces neutralizing antibodies against A subunit)

IT **Mutation**
(site-specific, Escherichia coli **detoxified** enterotoxin deriv. induces neutralizing antibodies against A subunit)

L3 ANSWER 9 OF 15 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1994:75437 CAPLUS
DOCUMENT NUMBER: 120:75437
TITLE: Genetic detoxification of **pertussis toxin** for vaccine
INVENTOR(S): Klein, Michel H.; Boux, Heather A.; Cockle, Stephen A.; Loosmore, Sheena M.; Zealey, Gavin R.
PATENT ASSIGNEE(S): Connaught Laboratories Ltd., Can.
SOURCE: U.S., 46 pp. Cont-in-part of U.S. 5,085,862.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 5244657	A	19930914	US 90-589423	19900928
US 5085862	A	19920204	US 88-275376	19881123
US 5221618	A	19930622	US 91-767837	19910930
US 5332583	A	19940726	US 91-788314	19911105
US 5358868	A	19941025	US 91-788313	19911105
US 5433945	A	19950718	US 92-979798	19921120
PRIORITY APPLN. INFO.:			GB 87-27489	19871124
			US 88-275376	19881123
			US 90-589423	19900928

AB A method is described for the prepn. of a safe, immunogenic, and efficacious vaccine for protection against pertussis. Specific functional sites of **pertussis toxin** have been identified, and, using this information, defined mutant holotoxins have been produced by site-directed mutagenesis of the toxin gene. A no. of these holotoxin analogs are detoxified, retain an immunodominant S1 epitope, are immunogenic, and are protective in the std. pertussis vaccine potency test in mice. The site of interaction of the S1 subunit with NAD was also detd.

IT Plasmid and Episome
(S-3319-3-9 and others, in mutant **pertussis toxin** prodn. vaccine in relation to)

IT Vaccines
(**mutant pertussis toxins** for, **pertussis toxin detoxification** in relation to)

IT Protein sequences
(of **pertussis toxin** mutants)

IT Toxins
RL: BIOL (Biological study)
(pertussis, mutants, for vaccine)

IT 152479-43-1, [.DELTA.9]-**Pertussis toxin** mutant (Bordetella pertussis 10536 clone S-2679-1-11 S1 subunit)
152479-44-2, [Glu9]-**pertussis toxin** mutant (Bordetella pertussis 10536 clone S-2815-1-8 S1 subunit)
152479-45-3, [Lys9]-**pertussis toxin** mutant (Bordetella pertussis 10536 clone S-2953-21 S1 subunit)
152479-46-4, [His9]-**pertussis toxin** mutant (Bordetella pertussis 10536 clone S-3046-4 S1 subunit)
152479-47-5, [.DELTA.13]-**Pertussis toxin** mutant (Bordetella pertussis 10536 clone S-2679-2-1 S1 subunit)
152479-48-6, [Glu13]-**pertussis toxin** mutant (Bordetella pertussis 10536 clone S-2779-2-1 S1 subunit)
152479-49-7, [.DELTA.9-13]-**Pertussis toxin** mutant (Bordetella pertussis 10536 clone S-2829-2-19 S1 subunit)
152479-50-0, [Glu9Glu13]-**pertussis toxin** mutant (Bordetella pertussis 10536 clone S-2779-3-2 S1 subunit)
152479-51-1, [Glu58]-**pertussis toxin** mutant

(Bordetella pertussis 10536 clone J-444-2-2 S1 subunit)
152479-52-2, [.DELTA.57.DELTA.58]-**Pertussis toxin**
mutant (Bordetella pertussis 10536 clone J-482-11 S1 subunit)
152479-53-3, [Ala26]-**pertussis toxin** mutant
(Bordetella pertussis 10536 clone S-3123-2 S1 subunit)
152479-54-4, [Cys26]-**pertussis toxin** mutant
(Bordetella pertussis 10536 clone S-3140-22 S1 subunit)
152479-55-5, [Ala41]-**pertussis toxin** mutant
(Bordetella pertussis 10536 clone S-2515-5-10 S1 subunit)
152479-56-6, [Ser41]-**pertussis toxin** mutant
(Bordetella pertussis 10536 clone S-3124-6 S1 subunit)
152479-57-7, [Ala201]-**pertussis toxin** mutant
(Bordetella pertussis 10536 clone S-2679-3-4 S1 subunit)
152479-58-8, [.DELTA.129]-**Pertussis toxin** mutant
(Bordetella pertussis 10536 clone S-2589-6 S1 subunit) 152479-59-9
, [Gly129]-**pertussis toxin** mutant (Bordetella
pertussis 10536 clone S-2515-3-6 S1 subunit) 152479-60-2,
[Gln129]-**pertussis toxin** mutant (Bordetella
pertussis 10536 clone S-2515-1-2 S1 subunit) 152479-61-3,
[Asp129]-**pertussis toxin** mutant (Bordetella
pertussis 10536 clone S-2515-2-4 S1 subunit) 152479-62-4,
[Asn129]-**pertussis toxin** mutant (Bordetella
pertussis 10536 clone S-2852-1-18 S1 subunit) 152479-63-5,
[Lys129]-**pertussis toxin** mutant (Bordetella
pertussis 10536 clone S-2515-4-11 S1 subunit) 152479-64-6,
[Arg129]-**pertussis toxin** mutant (Bordetella
pertussis 10536 clone M-32-2-4 S1 subunit) 152479-65-7, [His129]-
pertussis toxin mutant (Bordetella pertussis 10536
clone S-2937-1-2 S1 subunit) 152479-66-8, [Pro129]-
pertussis toxin mutant (Bordetella pertussis 10536
clone S-2959-2-28 S1 subunit) 152479-67-9, [Cys129]-
pertussis toxin mutant (Bordetella pertussis 10536
clone J-478-5 S1 subunit) 152479-68-0, [.DELTA.130]-
Pertussis toxin mutant (Bordetella pertussis 10536
clone S-2852-2-1 S1 subunit) 152479-69-1, [Phe130]-
pertussis toxin mutant (Bordetella pertussis 10536
clone S-2836-15 S1 subunit) 152479-70-4, [Gly129Ala130]-
pertussis toxin mutant (Bordetella pertussis 10536
clone S-2679-4-3 S1 subunit) 152479-71-5, [Gln129Ala130]-
pertussis toxin mutant (Bordetella pertussis 10536
clone M-38-1 S1 subunit) 152479-72-6, [Gly129Phe130]-
pertussis toxin mutant (Bordetella pertussis 10536
clone J-444-1-6 S1 subunit) 152479-73-7, [Gln10]-**pertussis**
toxin mutant (Bordetella pertussis 10536 clone S-2995-1-2 S3
subunit) 152479-74-8, [Asn92Arg93]-**pertussis**
toxin mutant (Bordetella pertussis 10536 clone S-2995-2-1 S3
subunit) 152479-75-9, [Asn105]-**pertussis toxin**
mutant (Bordetella pertussis 10536 clone S-2995-3-1 S3 subunit)
152479-76-0, [Ala41Ala201]-**pertussis toxin**

mutant (Bordetella pertussis 10536 clone S-2818-1 S1 subunit)
152479-77-1, [Ala41Gly129]-**pertussis toxin**
mutant (Bordetella pertussis 10536 clone S-2549-2 S1 subunit)
152479-78-2, [Glu9Gly129]-**pertussis toxin** mutant
(Bordetella pertussis 10536 clone M-45-1 S1 subunit) 152479-79-3,
[Glu9Gly129Ala130]-**pertussis toxin** mutant
(Bordetella pertussis 10536 clone S-2956-1 S1 subunit)
152479-80-6, [Glu13Gly129]-**pertussis toxin**
mutant (Bordetella pertussis 10536 clone S-2966-2-13 S1 subunit)
152479-81-7, [Glu13Gly129Ala130]-**pertussis toxin**
mutant (Bordetella pertussis 10536 clone S-2961-1 S1 subunit)
152479-82-8, [.DELTA.9Gln129]-**pertussis toxin**
mutant (Bordetella pertussis 10536 clone S-2730-1-1 S1 subunit)
152479-83-9, [.DELTA.9Gln129Ala130]-**pertussis**
toxin mutant (Bordetella pertussis 10536 clone S-2730-3-2 S1
subunit) 152479-84-0, [.DELTA.13Gln129]-**pertussis**
toxin mutant (Bordetella pertussis 10536 clone S-2730-2-1 S1
subunit) 152479-85-1, [.DELTA.13Gln129Ala130]-**pertussis**
toxin mutant (Bordetella pertussis 10536 clone S-2730-4-1 S1
subunit) 152479-86-2, [Lys13]-**pertussis toxin**
mutant (Bordetella pertussis 10536 clone JB-126-1-1 S1 subunit)
152479-87-3, [His58]-**pertussis toxin** mutant
(Bordetella pertussis 10536 clone S-3524-1 S1 subunit)
152479-88-4, [Lys58]-**pertussis toxin** mutant
(Bordetella pertussis 10536 clone S-3554-1-1 S1 subunit)
152479-89-5, [Ala35]-**pertussis toxin** mutant
(Bordetella pertussis 10536 clone S-3494-1 S1 subunit)
152479-90-8, [Ser129]-**pertussis toxin** mutant
(Bordetella pertussis 10536 clone S-3156-1-30 S1 subunit)
152479-91-9, [Ser130]-**pertussis toxin** mutant
(Bordetella pertussis 10536 clone S-3502-2-1 S1 subunit)
152479-92-0, [Glu58Gly129]-**pertussis toxin**
mutant (Bordetella pertussis 10536 clone S-3305-3 S1 subunit)
152479-93-1, [Lys9Gly129]-**pertussis toxin** mutant
(Bordetella pertussis 10536 clone S-3445-3-2 S1 subunit)
152479-94-2, [Lys9Glu58Gly129]-**pertussis toxin**
mutant (Bordetella pertussis 10536 clone S-3445-2-14 S1 subunit)
152479-95-3, [.DELTA.91-93]-**Pertussis toxin**
mutant (Bordetella pertussis 10536 clone S-3332-1-1 S3 subunit)
152479-96-4, [.DELTA.91-93]-**Pertussis toxin**
mutant (Bordetella pertussis 10536 clone S-3290-2-1 S2 subunit)
RL: BIOL (Biological study)
(amino acid sequence and residual toxicity of, detoxified
pertussis toxin for vaccine in relation to)
IT 103236-62-0, **Pertussis toxin** (Bordetella
pertussis 10536 clone J-169-1 S2 subunit) 152479-97-5,
Pertussis toxin (Bordetella pertussis 10536 clone
J-169-1 S1 subunit) 152479-98-6, **Pertussis toxin**
(Bordetella pertussis 10536 clone J-169-1 S3 subunit)

RL: BIOL (Biological study)
(amino acid sequence of and **mutants** of, **detoxified pertussis toxin** for vaccine in relation to)

IT 58319-92-9, ADP ribosyltransferase
RL: BIOL (Biological study)
(**pertussis toxin** mutants effect on activity of)

IT 51-45-6, Histamine, biological studies
RL: BIOL (Biological study)
(**pertussis toxin** mutants with decreased sensitivity activity of)

IT 53-84-9, NAD
RL: RCT (Reactant)
(photocrosslinking of, to **pertussis toxin** S1 subunit)

L3 ANSWER 10 OF 15 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1993:465358 CAPLUS
DOCUMENT NUMBER: 119:65358
TITLE: Characterization of **pertussis toxin** analogs containing mutations in B-oligomer subunits
AUTHOR(S): Loosmore, Sheena; Zealey, Gavin; Cockle, Stephen; Boux, Heather; Chong, Pele; Yacoob, Reza; Klein, Michel
CORPORATE SOURCE: Connaught Cent. Biotechnol. Res., Willowdale, ON, M2R 3T4, Can.
SOURCE: Infect. Immun. (1993), 61(6), 2316-24
CODEN: INFIBR; ISSN: 0019-9567
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The S2, S3, and S4 subunit genes of **pertussis toxin** (**PT**) from Bordetella pertussis were subjected to site-directed mutagenesis, and the resultant **PT** analogs assayed for altered biol. properties. PT analogs S2(T91,R92,N93).DELTA. and S2(Y102A,Y103A) exhibited reduced binding to fetuin. Several PT analogs with mutations in the S2, S3, or S4 subunit showed reduced in vitro toxicity, as measured in the Chinese hamster ovary (CHO) cell clustering assay. In particular, PT analogs S3(Y82A) and S3(I91,Y92,K93).DELTA. retained .ltoreq.10% residual toxicity. These mutants also exhibited significantly lower mitogenic and hemagglutinating activities and reduced in vivo activities, as measured by the histamine sensitization and leukocytosis assays. The S4(K54A,K57A) PT analog had significantly reduced CHO cell clustering activity, though other biol. activities remained unaffected. PT analogs S1(E129G)/S3(Y82A) and S1(E129G)/S3(I91,Y92,K93).DELTA. displayed a cumulative effect of the S1 and S3 mutations for in vitro and in vivo toxic activities.

These PT analogs, as well as S1(R9K,E129G)/S3(K82A) and S1(R9K,E129G)/S3(I91,Y92,K93).DELTA., still expressed an epitope which elicits a neutralizing antitoxin antibody and were protective in the mouse intracerebral challenge test. Recombinant pertussis vaccines based on PT analogs with **detoxifying mutations** in multiple subunits may thus represent the next generation of improved whooping cough vaccines.

IT Mutation
(in B-oligomer subunits of **pertussis toxin** analogs, characterization of)

IT Toxins
RL: BIOL (Biological study)
(pertussis, analogs, mutations in B-oligomer subunits, characterization of)

L3 ANSWER 11 OF 15 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1992:544511 CAPLUS
DOCUMENT NUMBER: 117:144511
TITLE: Construction of Bordetella pertussis strains that overproduce genetically inactivated **pertussis toxin**
AUTHOR(S): Zealey, Gavin; Loosmore, Sheena; Yacoob, Reza; Cockle, Stephen; Herbert, Andy; Klein, Michel
CORPORATE SOURCE: Res. Connaught Lab., Connaught Cent. Biotechnol., Willowdale, ON, M2R 3T4, Can.
SOURCE: Vaccines 92: Mod. Approaches New Vaccines Incl. Prev. AIDS [Annu. Meet.], 9th (1992), 367-71. Editor(s): Brown, Fred. Cold Spring Harbor Lab. Press: Cold Spring Harbor, N. Y.
CODEN: 57WXAL
DOCUMENT TYPE: Conference
LANGUAGE: English

AB **Pertussis toxin** (**PT**) is a major protective antigen in pertussis vaccines. For max. immunogenicity and safety, PT should be detoxified by genetic rather than chem. means. Such detoxification has been achieved by site-directed mutagenesis of the tox operon and prodn. of nontoxic PT analogs by recombinant Bordetella pertussis strains (M. A. Pizza et al. 1989, S. M. Loosmore et al. 1990). One highly **detoxified** PT analog contains the **mutations** Arg-9.fwdarw.Lys and Glu-129.fwdarw.Gly in subunit S1. This mol. is immunogenic and protective and is an appropriate antigen for inclusion in an acellular whooping cough vaccine. However, the relatively low level of PT secretion by B. pertussis is a limiting factor in the prodn. of such analogs. To increase the secretion of the Lys9Gly129 PT analog by B. pertussis, addnl. copies of the mutated tox operon were integrated at the tox and fha loci by unmarked allelic exchange. The resulting recombinant strains secrete amts. of PT analog proportional to gene dosage, and yields of up to 80 mg/L can be

obtained in 10-L fermentors.

IT Toxins
RL: BIOL (Biological study)
(pertussis, overprodn. of inactivated, by recombinant Bordetella pertussis)

L3 ANSWER 12 OF 15 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1991:406584 CAPLUS
DOCUMENT NUMBER: 115:6584
TITLE: Detoxification of **pertussis toxin** by site-directed mutagenesis: a review of Connaught strategy to develop a recombinant pertussis vaccine
AUTHOR(S): Loosmore, Sheena; Cockle, Stephen; Zealey, Gavin; Boux, Heather; Phillips, Kimberley; Fahim, Raafat; Klein, Michel
CORPORATE SOURCE: Connaught Cent. Biotechnol., Willowdale, ON, M2R 3T4, Can.
SOURCE: Mol. Immunol. (1991), 28(3), 235-8
CODEN: MOIMD5; ISSN: 0161-5890
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The authors identified and mutated several crit. amino acid residues in subunit S1 of **pertussis toxin** to generate highly detoxified **PT** analogs. Several of these analogs were scaled-up, purified, tested as potential vaccine candidates in exptl. animals, and found to be both immunogenic and protective. These **pertussis toxin** analogs, or derivs. thereof, will serve to design the new generation of recombinant acellular pertussis vaccines.
IT Vaccines
(for **pertussis**, detoxification of **pertussis toxin** by site-directed mutagenesis in development of)
IT Detoxication
(of **pertussis toxin**, by site-directed mutagenesis, in vaccine development)
IT Bordetella **pertussis**
(vaccine against, **toxin detoxification** by site-directed **mutagenesis** in development of)
IT Mutation
(site-specific, of **pertussis toxin**, in vaccine development)
IT 58319-92-9, ADP-Ribosyltransferase
RL: BIOL (Biological study)
(of **pertussis toxin** analogs, in vaccine development)
IT 51-45-6, Histamine, biological studies
RL: BIOL (Biological study)
(sensitization by, **pertussis toxin** analogs

induction of, in vaccine development)

L3 ANSWER 13 OF 15 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1991:241885 CAPLUS
DOCUMENT NUMBER: 114:241885
TITLE: Gene replacement in Bordetella pertussis by transformation with linear DNA
AUTHOR(S): Zealey, G. R.; Loosmore, S. M.; Yacoob, R. K.; Cockle, S. A.; Boux, L. J.; Miller, L. D.; Klein, M. H.
CORPORATE SOURCE: Connaught Cent. Biotechnol. Res., Willowdale, ON, M2R 3T4, Can.
SOURCE: Bio/Technology (1990), 8(11), 1025-9
CODEN: BTCHDA; ISSN: 0733-222X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The wild-type TOX operon of B. pertussis was replaced with in vitro **mutated, detoxified** alleles by electroporetic transformation using unmarked linear DNA. Uptake of DNA was selected by transient ampicillin resistance. Two simultaneous recombination events resulted in gene-replacement at the natural locus without integration of heterologous DNA. TOX Alleles were stable without selection and recombinant strains secreted non-toxic, fully assembled, protective **pertussis toxin** (**PT**) analogs with kinetics similar to the parental vaccine strain under prodn.-scale fermn. conditions. Strains generated in this way are suitable for the prodn. of recombinant whole-cell or component whooping cough vaccines that require no chem. modification of PT.

IT Transformation, genetic
(electroporation-mediated, of Bordetell **pertussis**, **pertussis toxin** gene replacement by, vaccine development in relation to)

IT Gene and Genetic element, microbial
RL: BIOL (Biological study)
(for **pertussis toxin**, of Bordetella pertussis, electroporation-mediated substitution of detoxified alleles for, vaccine development in relation to)

IT Vaccines
(non-toxic protective **pertussis toxin** analogs for, gene replacement in Bordetella pertussis in relation to)

IT Fermentation
(of recombinant Bordetell pertussis, for prodn. of recombinant non-toxic **pertussis toxin** analogs, for vaccine prodn.)

IT Bordetella pertussis
(TOX operon of, electroporation-mediated substitution of, detoxified alleles in, vaccine prodn. in relation to)

IT Toxins

RL: BIOL (Biological study)
(pertussis, operon for, of Bordetella pertussis, electroporation-mediated replacement of, vaccine development in relation to)

IT Operon
(tox, for **pertussis toxin**, of Bordetella pertussis, electroporetic transformation for replacement of, vaccine development in relation to)

L3 ANSWER 14 OF 15 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1990:629139 CAPLUS
DOCUMENT NUMBER: 113:229139
TITLE: Engineering of genetically detoxified **pertussis toxin** analogs for development of a recombinant whooping cough vaccine
AUTHOR(S): Loosmore, Sheena M.; Zealey, Gavin R.; Boux, Heather A.; Cockle, Stephen A.; Radika, Kesavan; Fahim, Raafat E. F.; Zobrist, Gloria J.; Yacoob, Reza K.; Chong, Pele C. S.; et al.
CORPORATE SOURCE: Connaught Cent. Biotechnol. Res., Willowdale, ON, M2R 3T4, Can.
SOURCE: Infect. Immun. (1990), 58(11), 3653-62
CODEN: INFIBR; ISSN: 0019-9567
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Pertussis toxin** (**PT**) is an important protective antigen in vaccines against whooping cough, and a genetically detoxified **PT** analog is the preferred form of the immunogen. Several amino acids of the S1 subunit were identified as functionally crit. residues by site-directed mutagenesis, specifically, those at positions 9, 13, 26, 35, 41, 58, and 129. Eighty-three mutated **PT** operons were introduced into Bordetella parapertussis, and the resultant **toxin** analogs were screened for expression levels, enzymic activity, residual toxicity, and antigenicity. While more than half of the mutants were poorly secreted or assembled, the rest were fully assembled and most were highly detoxified. Single mutations resulted in up to a 1,000-fold redn. in both toxic and enzymic activities, while PT analogs with multiple mutations (Lys-9 Gly-129, Glu-58 Gly-129, and Lys-9 Glu-58 Gly-129) were 106-fold detoxified. Operons coding for stable and nontoxic mutants shown to express a crit. immunodominant protective epitope were returned to the chromosome of B. pertussis by allelic exchange. In vivo anal. of the toxin analogs showed a dramatic redn. in histamine sensitization and lymphocytosis-promoting activities, paralleling the redn. in toxic activities. All mutants were protective in an intracerebral challenge test, and the Lys-9 Gly-129 analog was more immunogenic than the toxoid. PT analogs such as those described represent

suitable components for the design of a recombinant whooping cough vaccine.

IT Vaccines
(against whooping cough, recombinant detoxified **pertussis toxin** analogs as)

IT **Mutation**
(in **detoxified** pertussis analogs prepn.)

IT Detoxication
(of **pertussis toxin** recombinant analogs, in vaccine prepn.)

IT Whooping cough
(vaccine against, recombinant detoxified **pertussis toxin** analogs as)

IT Toxins
RL: BIOL (Biological study)
(pertussis, recombinant analogs of, as vaccine against whooping cough)

L3 ANSWER 15 OF 15 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1990:404262 CAPLUS
DOCUMENT NUMBER: 113:4262
TITLE: Detoxification of **pertussis toxin** by site-directed mutagenesis
AUTHOR(S): Cockle, S.; Loosmore, S.; Radika, K.; Zealey, G.; Boux, H.; Phillips, K.; Klein, M.
CORPORATE SOURCE: Connaught Res. Inst., Willowdale, ON, M2R 3T4, Can.
SOURCE: Adv. Exp. Med. Biol. (1989), 251(Immunobiol. Proteins Pept. 5), 209-14
CODEN: AEMBAP; ISSN: 0065-2598
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A series of pertussis holotoxin (PT) analogs was generated in engineered Bordetella strains by mutation of the catalytic S1 subunit at 7 crit. sites, including Glu129, which was found by photocross-linking to be near the active site for NAD hydrolysis. Many of these **mutants** were highly **detoxified** according to CHO cell clustering and ADPR assays, and retained an immunodominant protective S1 epitope. Three affinity-purified analogs were immunogenic and protective in mice, yet exhibited low toxicity. This is an important advance towards the development of a genetically inactivated form of PT for inclusion in a nwe generation of whooping cough vaccines.

IT Vaccines
(for **pertussis, pertussis toxin detoxification** by site-directed **mutagenesis** in relation to)

IT Detoxication
(of **pertussis toxin**, by site-directed

mutagenesis, vaccine in relation to)
IT Toxins
RL: BIOL (Biological study)
(pertussis, site-directed mutations in, vaccine toxicity in relation to)
IT Mutation
(site-specific, of **pertussis toxin**, vaccine in relation to)

=> d his l4-; d 1-19 ibib abs

(FILE 'MEDLINE, BIOSIS, EMBASE, TOXLIT, TOXLINE, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, CABA, AGRICOLA' ENTERED AT 15:21:16 ON 27 JUL 1999)
L4 37 S L3
L5 19 DUP REM L4 (18 DUPLICATES REMOVED)

L5 ANSWER 1 OF 19 TOXLIT
ACCESSION NUMBER: 1998:68701 TOXLIT
DOCUMENT NUMBER: CA-128-279567V
TITLE: Immunogenic **detoxified mutant** Escherichia **coli LT**-A **toxin**.
AUTHOR: Pizza M; Giuliani MM; Rappuoli R
SOURCE: (1998). PCT Int. Appl. PATENT NO. 9818928 05/07/1998 (Rappuoli, Rino).
CODEN: PIXXD2.
PUB. COUNTRY: ITALY
DOCUMENT TYPE: Patent
FILE SEGMENT: CA
LANGUAGE: English
OTHER SOURCE: CA 128:279567
ENTRY MONTH: 199806
AB An immunogenic detoxified protein is provided which comprises the amino acid sequence of subunit A of an E. **coli** heat labile **toxin** (**LT**-A) or a fragment thereof in which at least amino acid Ala-72 of the A subunit is mutated, preferably by substitution with Arg. The toxoid is useful as vaccine against an enterotoxigenic strain of E. coli and is produced by recombinant DNA means by site-directed mutagenesis. A 1.5 kb SmaI-EcoRI fragment from plasmid pEWD299 contg. the gene for **LT**-A and the **LT** promoter region was subcloned to produce vector BS-**LT**-A. BS-**LT**-A was mutagenized with oligonucleotide oligoLT-A72R to change the Ala-72 codon to the Arg codon and ligated to the EcoRI-HindIII fragment contg. the gene for **LT**-B and cloned to produce vector BS-LTA72R. E. coli was transformed with BS-LTA72R and the **LT**-A72R mutant purified. ADP-ribosylation of **LT**-A72R was lower than wild type **LT**-A, the toxicity of **LT**-A72R was 10-5 lower than

wild type **LT**-A, and **LT**-A72R proved to be an effective mucosal adjuvant.

L5 ANSWER 2 OF 19 WPIDS COPYRIGHT 1999 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1999-070064 [06] WPIDS
DOC. NO. CPI: C99-020598
TITLE: **Detoxified mutants** of bacterial **ADP-ribosylating toxins** as parenteral adjuvants - useful to enhance humoral and cell-mediated immune responses in vertebrates when administered with selected antigen e.g. in disease treatment.
DERWENT CLASS: B04 D16
INVENTOR(S): BARCHFELD, G; DEL GIUDICE, G; RAPPUOLI, R
PATENT ASSIGNEE(S): (CHIR) CHIRON CORP
COUNTRY COUNT: 80
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9842375	A1	981001	(9906)*	EN	51

RW: AT BE CH DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM GW HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG UZ VN YU ZW

AU 9865713 A 981020 (9909)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9842375	A1	WO 98-US5454	980319
AU 9865713	A	AU 98-65713	980319

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9865713	A	Based on	WO 9842375

PRIORITY APPLN. INFO: US 98-44696 980318; US 97-41227 970321
AN 1999-070064 [06] WPIDS
AB WO 9842375 A UPAB: 19990210
A parenteral adjuvant composition is new, comprising a **detoxified mutant** of a bacterial **ADP-ribosylating toxin** as the parenteral adjuvant, at least one selected antigen and optionally a pharmaceutically

acceptable (optionally topical) vehicle. In the disclosure '**Detoxified' mutants** are defined as completely non-toxic/having low residual toxicity (preferably less than 0.01 % of natural counterparts) as measured by e.g. morphological changes induced in Y1 cells.

USE - The adjuvant composition can be administered parenterally in conjunction with at least one antigen in methods to immunise vertebrate subjects (claimed). The adjuvant has the ability to enhance the humoral and cell-mediated immune responses elicited by the antigen (e.g. by making the antigen more strongly immunogenic or necessitating fewer/lower antigen doses). It can be administered prior/subsequent to the antigen, and is preferably administered within a short space of time to the same site; it can also be administered in isolation from antigens as a boost following systemic or mucosal antigen administration.

Most preferably, the adjuvant is co-administered with the antigen in the compositions and a pharmaceutically acceptable carrier. The antigen may be derived from viruses, bacteria, parasites and fungi or may be tumour antigens, self-antigens and allergens. The compositions are therefore useful in the treatment and prevention of e.g. viral diseases, allergic manifestations, diseases caused by pathogens (e.g. bacteria or parasites), AIDS, autoimmune diseases (e.g. Systemic Lupus Erythematosus), Alzheimer's disease and cancers. The adjuvant can also be used to prepare antibodies against selected antigen(s), useful e.g. for diagnostic purposes or for antigen purification. The composition of can also be used to manufacture medicaments useful for parenterally immunising (e.g. subcutaneously, intramuscularly or especially transcutaneously) vertebrates (claimed).

ADVANTAGE - The adjuvant compositions function when administered parenterally, so allow immunity to be conferred to substances not amenable to other modes of administration (e.g. oral or intranasal delivery).

Dwg.0/2

L5 ANSWER 3 OF 19 MEDLINE DUPLICATE 1

ACCESSION NUMBER: 1998347282 MEDLINE

DOCUMENT NUMBER: 98347282

TITLE: Mucosal immunogenicity of genetically detoxified derivatives of heat labile toxin from Escherichia coli.

AUTHOR: Douce G; Giuliani M M; Giannelli V; Pizza M G; Rappuoli R; Dougan G

CORPORATE SOURCE: Department of Biochemistry, Imperial College of Science, Technology and Medicine, London, UK.

SOURCE: VACCINE, (1998 Jul) 16 (11-12) 1065-73. Journal code: X6O. ISSN: 0264-410X.

PUB. COUNTRY: ENGLAND: United Kingdom

Journal; Article; (JOURNAL ARTICLE)

Searcher : Shears 308-4994

LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199812
ENTRY WEEK: 19981204

AB Using a fixed dose of antigen, the immune response to **detoxified mutants** of **LT**-WT following intranasal (i.n.), subcutaneous (s.c.) and oral (i.g.) immunisation has been studied. When given i.n., both **LT**-WT and mutant **toxin**, K63, generated significant levels of **toxin**-specific IgG in the serum, and the levels of IgA in nasal and lung lavages were greater than those induced by rLT-B. In comparison, i.g. immunisation of mice with a similar quantity of either **LT**-WT or K63 **toxin** induced barely detectable levels of IgG in the sera. However, if the amount of protein used for i.g. immunisation was increased tenfold, relatively good levels of **toxin**-specific IgG were induced in the sera by both **LT**-WT or K63. Low levels of **toxin**-specific IgA were also observed in intestinal washes from these mice. Western blotting of the sera, using the native **toxin** as an antigen, demonstrated the presence of both anti-A and anti-B subunit antibodies. Most significantly, **toxin**-neutralising antibodies were induced in the serum, with the strongest activity being induced by the **LT**-WT, an intermediate activity induced by mutant K63 and a lower response by rLT-B. Together, these data show that ADP-ribosyltransferase is not necessary for mucosal immunogenicity of these proteins, and that the i.n. route of immunisation is more effective than the i.g. route of immunisation for the generation of both systemic (IgG) and mucosal (IgA) immune responses.

L5 ANSWER 4 OF 19 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 1998307520 MEDLINE
DOCUMENT NUMBER: 98307520
TITLE: **Pertussis toxin** potentiates Th1 and Th2 responses to co-injected antigen: adjuvant action is associated with enhanced regulatory cytokine production and expression of the co-stimulatory molecules B7-1, B7-2 and CD28.
AUTHOR: Ryan M; McCarthy L; Rappuoli R; Mahon B P; Mills K H
CORPORATE SOURCE: Department of Biology, National University of Ireland, Maynooth, Co. Kildare.
SOURCE: INTERNATIONAL IMMUNOLOGY, (1998 May) 10 (5) 651-62. Journal code: AY5. ISSN: 0953-8178.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199811
ENTRY WEEK: 19981102

AB **Pertussis toxin** (**PT**) is a major virulence factor of Bordetella pertussis which exerts a range of effects on the immune system, including the enhancement of IgE, IgA and IgG production, delayed-type hypersensitivity reactions, and the induction of experimental autoimmune diseases. However, the mechanism by which **PT** mediates adjuvanticity remains to be defined. In this investigation we have shown that **PT** can potentiate antigen-specific T cell proliferation and the secretion of IFN-gamma, IL-2, IL-4 and IL-5 when injected with foreign antigens. A chemically **detoxified PT** and a genetic **mutant** with substitutions/deletions in the S-1 and B oligomer components that abrogate enzymatic and binding activity displayed no adjuvant properties. In contrast, a non-toxic S-1 mutant devoid of enzymatic activity but still capable of receptor binding retained its adjuvanticity, augmenting the activation of both Th1 and Th2 subpopulations of T cells. In an attempt to address the mechanism of T cell activation, we found that **PT** stimulated the production of IFN-gamma and IL-2 by naive T cells and IL-1 by macrophages. Therefore potentiation of distinct T cell subpopulations may have resulted in part from the positive influence of IFN-gamma on the development of Th1 cells and the co-stimulatory role of IL-1 for Th2 cells. Furthermore, **PT** augmented expression of the co-stimulatory molecules B7-1 and B7-2 on macrophages and B cells, and CD28 on T cells, suggesting that the adjuvant effect may also be associated with facilitation of the second signal required for maximal T cell activation. This study demonstrates that the immunopotentiating properties of **PT** are largely independent of ADP-ribosyltransferase activity, but are dependent on receptor binding activity and appear to involve enhanced activation of T cells.

L5 ANSWER 5 OF 19 EMBASE COPYRIGHT 1999 ELSEVIER SCI. B.V.

ACCESSION NUMBER: 1998074807 EMBASE

TITLE: Recent advances in immunological adjuvants: The development of particulate antigen delivery systems.

AUTHOR: O'Hagan D.T.

CORPORATE SOURCE: D.T. O'Hagan, Chiron Corporation, 4560 Horton Street, Emeryville, CA 94704, United States

SOURCE: Expert Opinion on Investigational Drugs, (1998) 7/3 (349-359).
Refs: 70
ISSN: 1354-3784 CODEN: EOIDER

COUNTRY: United Kingdom

DOCUMENT TYPE: Journal; General Review

FILE SEGMENT: 004 Microbiology
026 Immunology, Serology and Transplantation
030 Pharmacology
037 Drug Literature Index
039 Pharmacy

Searcher : Shears 308-4994

LANGUAGE: English
SUMMARY LANGUAGE: English
AB New generation vaccines, including those based on recombinant proteins, are safer than traditional vaccines, but are less immunogenic. Therefore, there is an urgent need for the development of new and improved vaccine adjuvants. A number of potent immunostimulatory molecules obtained from bacterial cells or plants have been extensively evaluated as adjuvants. However, a number of these molecules have displayed significant toxicity, both in preclinical animal models and in human clinical trials. An alternative approach to the development of novel adjuvants involves the preparation of particulate antigen delivery systems of similar dimensions to natural pathogens. In the absence of additional immunostimulatory molecules, emulsion droplets and microparticles have been shown to be potent adjuvants for the induction of both humoral and cell-mediated immune responses following systemic administration. Moreover, particulate delivery systems have been shown to display an acceptable toxicity profile in a number of clinical trials. Particulate antigen delivery systems also have the potential to function as potent adjuvants following administration by mucosal routes, including oral and intranasal. An alternative approach to the mucosal delivery of vaccines involves the use of genetically **detoxified mutant toxins**, e.g., **LT**-K63, as mucosal adjuvants. The use of novel adjuvants and antigen delivery systems is likely to extend the use of vaccines into the area of therapeutics, involving the eradication of infectious diseases and cancers, or the amelioration of autoimmune disorders.

L5 ANSWER 6 OF 19 EMBASE COPYRIGHT 1999 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 1998243582 EMBASE
TITLE: Eradication of chronic Helicobacter pylori infection by therapeutic vaccination.
AUTHOR: Crabtree J.E.
CORPORATE SOURCE: J.E. Crabtree, Molecular Medicine Unit, Clinical Sciences Building, St James's University Hospital, Leeds LS9 7TF, United Kingdom
SOURCE: Gut, (1998) 43/1 (7-8).
Refs: 24
ISSN: 0017-5749 CODEN: GUTTAK
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; (Short Survey)
FILE SEGMENT: 004 Microbiology
026 Immunology, Serology and Transplantation
030 Pharmacology
037 Drug Literature Index
048 Gastroenterology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Chronic infection of the gastroduodenal mucosae by the gram-negative spiral bacterium Helicobacter pylori is responsible for chronic active gastritis, peptic ulcers, and gastric cancers such as adenocarcinoma and low- grade B-cell lymphoma. The success of eradication by antibiotic therapy is being rapidly hampered by the increasing occurrence of antibiotic-resistant strains. An attractive alternative approach to combat this infection is represented by the therapeutic use of vaccines. In the present work, we have exploited the mouse model of persistent infection by mouse-adapted H. pylori strains that we have developed to assess the feasibility of the therapeutic use of vaccines against infection. We report that an otherwise chronic H. pylori infection in mice can be successfully eradicated by intragastric vaccination with H. pylori antigens such as recombinant VacA and CagA, which were administered together with a genetically **detoxified mutant** of the heat-labile exterotoxin of Escherichia coli (referred to as LTK63), in which the serine in position 63 was replaced by a lysine. Moreover, we show that therapeutic vaccination confers efficacious protection against reinfection. These results represent strong evidence of the feasibility of therapeutic use of VacA- or CagA-based vaccine formulations against H. pylori infection in an animal model and give substantial preclinical support to the application of this kind of approach in human clinical trials.

L5 ANSWER 7 OF 19 WPIDS COPYRIGHT 1999 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1997-424757 [39] WPIDS
DOC. NO. CPI: C97-135893
TITLE: Immunogenic **detoxified mutants** of **cholera toxin** - produced by substitution of at least one amino acid, gives a toxin with lower toxicity than natural toxin, used in cholera vaccine.
DERWENT CLASS: B04 D16
INVENTOR(S): FONTANA, M R; PIZZA, M; RAPPUOLI, R
PATENT ASSIGNEE(S): (CHIR-N) CHIRON SPA
COUNTRY COUNT: 21
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9729771	A1	970821	(9739)*	EN	54
RW: AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE					
W: CA JP US					
EP 880361	A1	981202	(9901)	EN	
R: AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE					

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE

WO 9729771	A1	WO 97-IB183	970217
EP 880361	A1	EP 97-902552	970217
		WO 97-IB183	970217

FILING DETAILS:

PATENT NO	KIND		PATENT NO
EP 880361	A1	Based on	WO 9729771

PRIORITY APPLN. INFO: GB 96-3314 960216
AN 1997-424757 [39] WPIDS
AB WO 9729771 A UPAB: 19970926
A novel immunogenic detoxified protein (I) comprises the amino acid sequence of subunit A of a **cholera toxin** (**CT**-A) or its fragment, in which at least amino acid is substituted by another amino acid. Purified (I) has a residual toxicity which is greater than 10000 times lower than the toxicity of its natural counterpart.

USE - (I) is used as a vaccine against Vibrio cholerae in mammals (claimed).

ADVANTAGE - The vaccine provides longer lasting protection than the conventional vaccine of killed bacteria, without special side effects.

Dwg.0/0

L5 ANSWER 8 OF 19 WPIDS COPYRIGHT 1999 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1997-108961 [10] WPIDS
DOC. NO. CPI: C97-034829
TITLE: New immunogenic detoxified proteins - comprising **cholera toxin** A or E. **coli** heat labile **toxin** A sub-unit with substitutions at Ser-63 and Arg-192..
DERWENT CLASS: B04 D16
INVENTOR(S): FONTANA, M R; GIANNELLI, V; PIZZA, M; RAPPUOLI, R
PATENT ASSIGNEE(S): (BIOC-N) BIOCINE SPA; (CHIR) CHIRON SPA
COUNTRY COUNT: 22
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9702348	A1	970123	(9710)*	EN	64
RW: AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE					
W: AU CA JP US					
AU 9662388	A	970205	(9721)		
EP 835314	A1	980415	(9819)	EN	
R: AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE					

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9702348	A1	WO 96-IB703	960701
AU 9662388	A	AU 96-62388	960701
EP 835314	A1	EP 96-921043	960701
		WO 96-IB703	960701

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9662388	A	Based on	WO 9702348
EP 835314	A1	Based on	WO 9702348

PRIORITY APPLN. INFO: GB 95-13371 950630
AN 1997-108961 [10] WPIDS
AB WO 9702348 A UPAB: 19990316
An immunogenic detoxified protein (IDP) is claimed, comprising the amino acid sequence of subunit A of a **cholera toxin** (**CT**-A) or a fragment of the amino acid sequence of subunit A of an E. **coli** heat labile **toxin** (**LT**-A) or a fragment where the amino acids at, or in positions corresponding to, Ser-63 and Arg-192 are replaced with another amino acid. Also claimed are: (1) a DNA sequence encoding an IDP as above; (2) a vector carrying a DNA as in (1); and (3) a host cell line transformed with a vector as in (2).

USE - The IDPs can be administered for the prevention or treatment of a disease caused by Vibrio cholerae or an enterotoxigenic strain of E. coli. They can also be used as mucosal adjuvants for other immunogenic proteins

ADVANTAGE - The **mutation** at Ser-63 **detoxifies** the toxins while the **mutation** at Arg-192 markedly improves the stability of the resulting protein.

Dwg.0/7

L5 ANSWER 9 OF 19 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1994:325732 BIOSIS
DOCUMENT NUMBER: PREV199497338732
TITLE: Characterization of PT analogs with **detoxifying mutations** in the B subunit genes.
AUTHOR(S): Loosmore, S.; Zealey, G.; Cockle, S.; Boux, H.; Yacoob, R.; Klein, M.
CORPORATE SOURCE: Connaught Cent. Biotechnol. Res., 1755 Steeles Ave. W., Willowdale, ON M2R 3T4 Canada
SOURCE: Freer, J. [Editor]; Aitken, R. [Editor]; Alouf, J. E. [Editor]; Boulnois, G. [Editor]. FEMS Symposium,

(1994) No. 73, pp. 408-409. FEMS Symposium; Bacterial protein toxins.
Publisher: Gustav Fischer Verlag Wollgrasweg 49, D-7000 Stuttgart, Germany.
Meeting Info.: Sixth European Workshop Stirling, Scotland, UK June 27-July 2, 1993
ISSN: 0163-9188. ISBN: 3-437-11535-9, 1-56081-385-7.
DOCUMENT TYPE: Book; Conference
LANGUAGE: English

L5 ANSWER 10 OF 19 WPIDS COPYRIGHT 1999 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1993-227320 [28] WPIDS
DOC. NO. CPI: C93-101279
TITLE: Immunogenic **detoxified mutant cholera toxin** and heat labile toxin - useful as vaccines against infection by Vibrio cholerae and enterotoxin producing Escherichia coli.
DERWENT CLASS: B04 D16
INVENTOR(S): DOMENIGHINI, M; HOL, W; PIZZA, M; RAPPUOLI, R
PATENT ASSIGNEE(S): (BIOC-N) BIOCINE SCLAVO SPA; (BIOC-N) BIOCINE SPA; (CHIR) CHIRON SPA; (CHIR-N) CHIRON SPA; (ISTS) SCLAVO RICERCA SRL
COUNTRY COUNT: 42
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9313202	A1	930708	(9328)*	EN	60
RW: AT BE CH DE DK ES FR GB GR IE IT LU MC NL OA PT SE					
W: AT AU BB BG BR CA CH CS DE DK ES FI GB HU JP KP KR LK LU MG MN MW NL NO NZ PL PT RO RU SD SE US					
AU 9333476	A	930728	(9347)		
EP 620850	A1	941026	(9441)	EN	
R: AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE					
TW 239146	A	950121	(9515)		
JP 07506240	W	950713	(9536)		20
IT 1253009	B	950710	(9608)		
SG 48217	A1	980417	(9828)		
EP 869181	A1	981007	(9844)	EN	
R: AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE					
EP 620850	B1	990303	(9913)	EN	
R: AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE					
DE 69228563	E	990408	(9920)		
ES 2127808	T3	990501	(9924)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE

WO 9313202	A1		WO 92-EP3016	921230
AU 9333476	A		AU 93-33476	921230
EP 620850	A1		WO 92-EP3016	921230
			EP 93-902138	921230
TW 239146	A		TW 93-100298	930118
JP 07506240	W		WO 92-EP3016	921230
			JP 93-511447	921230
IT 1253009	B		IT 91-MI3513	911231
SG 48217	A1		SG 96-8033	921230
EP 869181	A1	Div ex	EP 93-902138	921230
			EP 98-200534	921230
EP 620850	B1		WO 92-EP3016	921230
			EP 93-902138	921230
		Related to	EP 98-200534	921230
DE 69228563	E		DE 92-628563	921230
			WO 92-EP3016	921230
			EP 93-902138	921230
ES 2127808	T3		EP 93-902138	921230

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9333476	A	Based on	WO 9313202
EP 620850	A1	Based on	WO 9313202
JP 07506240	W	Based on	WO 9313202
EP 869181	A1	Div ex	EP 620850
EP 620850	B1	Related to	EP 869181
		Based on	WO 9313202
DE 69228563	E	Based on	EP 620850
		Based on	WO 9313202
ES 2127808	T3	Based on	EP 620850

PRIORITY APPLN. INFO: IT 91-MI3513 911231
AN 1993-227320 [28] WPIDS
AB WO 9313202 A UPAB: 19990316
An immunogenic detoxified protein (I) comprises (a) the amino acid sequence of subunit (A) of a **cholera toxin** (**CT**-A) (fragment) or (b) the amino acid sequence of subunit A of an Escherichia **coli** heat labile **toxin** (**LT**-A) (fragment); where at least 1 amino acid(s) at or in positions Val-53, Ser-63, Val-97, Tyr-104, or Pro-106 are replaced with another amino acid.

Also new are: (1) an immunogenic compsn. for use as a vaccine comprising (I) and a pharmaceutically acceptable carrier; (2) the DNA sequence encoding (I); (3) a vector carrying the sequence of (2); (4) a host cell line transformed with the vector of (3); (5) prodn. of (I) comprising culturing the cell (4); (6) prodn. of the

DNA of (2) by subjecting a DNA encoding a CT-A or an LT-A (fragment) to site-directed mutagenesis; (7) the use of the vaccine of (1) to vaccinate a mammal against Vibrio cholerae or an enterotoxigenic strain of E.coli; and (8) a process for the formulation of the vaccine of (7).

USE/ADVANTAGE - (I) can be used to give total protection against cholera or enterotoxigenic E. coli. It retains immunogenic properties but has significantly reduced or absent toxicity

Dwg.0/3

L5 ANSWER 11 OF 19 MEDLINE DUPLICATE 3

ACCESSION NUMBER: 93273477 MEDLINE

DOCUMENT NUMBER: 93273477

TITLE: Characterization of **pertussis toxin** analogs containing mutations in B-oligomer subunits.

AUTHOR: Loosmore S; Zealey G; Cockle S; Boux H; Chong P; Yacoob R; Klein M

CORPORATE SOURCE: Connaught Centre for Biotechnology Research, Willowdale, Ontario, Canada..

SOURCE: INFECTION AND IMMUNITY, (1993 Jun) 61 (6) 2316-24. Journal code: GO7. ISSN: 0019-9567.

PUB. COUNTRY: United States

Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals; Cancer Journals

ENTRY MONTH: 199309

AB The S2, S3, and S4 subunit genes of **pertussis toxin** (**PT**) from Bordetella pertussis were subjected to site-directed mutagenesis, and the resultant **PT** analogs were assayed for altered biological properties. **PT** analogs S2(T91,R92,N93) delta and S2(Y102A,Y103A) exhibited reduced binding to fetuin. Several **PT** analogs with mutations in the S2, S3, or S4 subunit showed reduced in vitro toxicity, as measured in the Chinese hamster ovary (CHO) cell clustering assay. In particular, **PT** analogs S3(Y82A) and S3(I91,Y92,K93) delta retained 10% or less residual toxicity. These mutants also exhibited significantly lower mitogenic and hemagglutinating activities and reduced in vivo activities, as measured by the histamine sensitization and leukocytosis assays. The S4(K54A,K57A) **PT** analog had significantly reduced CHO cell clustering activity, though other biological activities remained unaffected. **PT** analogs S1(E129G)/S3(Y82A) and S1(E129G)/S3(I91,Y92,K93) delta displayed a cumulative effect of the S1 and S3 mutations for both in vitro and in vivo toxic activities. These **PT** analogs, as well as S1(R9K,E129G)/S3(K82A) and S1(R9K,E129G)/S3(I91,Y92,K93) delta, still expressed an epitope which elicits a neutralizing antitoxin antibody and were protective in the mouse intracerebral challenge test. Recombinant pertussis vaccines based on **PT** analogs with **detoxifying**

mutations in multiple subunits may thus represent the next generation of improved whooping cough vaccines.

L5 ANSWER 12 OF 19 TOXLINE
ACCESSION NUMBER: 1994:56416 TOXLINE
DOCUMENT NUMBER: CRISP-94-E00518-03
TITLE: DEVELOPMENT OF DETOXIFIED **PERTUSSIS TOXIN** FOR ACELLULAR WHOOPING COUGH VACCINE.
AUTHOR: KEITH J M
CORPORATE SOURCE: NIDR, NIH
U.S. DEPT. OF HEALTH AND HUMAN SERVICES; PUBLIC HEALTH SERVICE; NATIONAL INST. OF HEALTH, NATIONAL INSTITUTE OF DENTAL RESEARCH.
CONTRACT NUMBER: Z01DE00518-03
SOURCE: (1992). Crisp Data Base National Institutes Of Health. Award Type: G = Grant
DOCUMENT TYPE: (RESEARCH)
FILE SEGMENT: CRISP
LANGUAGE: English
ENTRY MONTH: 199403
AB RPROJ/CRISP Whooping cough is caused by an infection of the respiratory tract with Bordetella pertussis bacteria. This disease is effectively controlled by the current vaccine which consists of killed whole B. pertussis cells. Though efficacious, the present vaccine produces unacceptable side effects. The major protective antigen in whooping cough vaccines is **pertussis toxin**. Clinical trials of acellular pertussis products strongly indicate that **pertussis toxin** will be a necessary and perhaps sufficient component of any new vaccine. Chemically "inactivated" **pertussis toxin** vaccines have been produced with reduced side effects and reasonable efficacy, however, residual activity may exist. Through our gene expression experiments we discovered a molecular approach for inactivation of **pertussis toxin**. Using site-specific DNA mutagenesis, the S1 subunit was modified by either a single or double amino acid substitution. These mutations virtually eliminated toxic activity, yet the immunogenic protective epitope was retained. We have devised several methods to transfer these genetic changes into the chromosome of B. pertussis, thus creating several new mutant strains. Using these new **mutant** strains, a genetically **detoxified pertussis toxin** molecule has been produced. This nontoxic holotoxin has strong immunoprotective properties and can be used as a vaccine antigen without chemical inactivation. Immunoprotein studies as well as characterization of the biological activities associated with these new strains are currently underway in our laboratory and at the National Institute of Health in Tokyo, Japan. In addition to this effort, new constructs have been produced to utilize a live Salmonella oral vaccine. These construct are being tested in an

animal model as a collaboration with researchers at Washington University in St. Louis and University of Missouri in Columbia.

L5 ANSWER 13 OF 19 TOXLIT
ACCESSION NUMBER: 1992:98133 TOXLIT
DOCUMENT NUMBER: CA-117-144511Z
TITLE: Construction of Bordetella pertussis strains that overproduce genetically inactivated **pertussis toxin**.
AUTHOR: Zealey G; Loosmore S; Yacoob R; Cockle S; Herbert A; Klein M
CORPORATE SOURCE: Res. Connaught Lab., Connaught Cent. Biotechnol., Willowdale
SOURCE: Vaccines 92: Mod. Approaches New Vaccines Incl. Prev. AIDS [Annu. Meet.], 9th, (1992). pp. 367-71. CODEN: 57WXAL.
PUB. COUNTRY: Canada
DOCUMENT TYPE: Book; (MONOGRAPH)
FILE SEGMENT: CA
LANGUAGE: English
OTHER SOURCE: CA 117:144511
ENTRY MONTH: 199211
AB **Pertussis toxin** (**PT**) is a major protective antigen in pertussis vaccines. For max. immunogenicity and safety, **PT** should be detoxified by genetic rather than chem. means. Such detoxification has been achieved by site-directed mutagenesis of the tox operon and prodn. of nontoxic **PT** analogs by recombinant Bordetella pertussis strains (M. A. Pizza et al. 1989, S. M. Loosmore et al. 1990). One highly **detoxified PT** analog contains the **mutations** Arg-9.fwdarw.Lys and Glu-129.fwdarw.Gly in subunit S1. This mol. is immunogenic and protective and is an appropriate antigen for inclusion in an acellular whooping cough vaccine. However, the relatively low level of **PT** secretion by B. pertussis is a limiting factor in the prodn. of such analogs. To increase the secretion of the Lys9Gly129 **PT** analog by B. pertussis, addnl. copies of the mutated tox operon were integrated at the tox and fha loci by unmarked allelic exchange. The resulting recombinant strains secrete amts. of **PT** analog proportional to gene dosage, and yields of up to 80 mg/L can be obtained in 10-L fermentors.

L5 ANSWER 14 OF 19 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1992:189143 BIOSIS
DOCUMENT NUMBER: BA93:100093
TITLE: EVALUATION OF ACELLULAR DPT VACCINES IN INFANTS.
AUTHOR(S): NENCIONI L; PODDA A; PEPPOLONI S; VOLPINI G; MARSILI I; CONTU B; COSSU M A; VANNI R; ET AL
CORPORATE SOURCE: R. RAPPUOLI, SCLAVO RES. CENT., VIA FIORENTIAN 1,

53100 SIENA, ITALY.
SOURCE: MEM INST BUTANTAN (SAO PAULO), (1991) 53 (SUPPL 1), 21-29.
CODEN: MIBUAH. ISSN: 0073-9901.
FILE SEGMENT: BA; OLD
LANGUAGE: English

AB Two acellular DPT vaccines containing, as pertussis components, the genetically **detoxified pertussis toxin mutant PT**-9K/129G, either alone or combined with FHA and 69K, were evaluated for safety and immunogenicity in infants 8-14 months old. Both vaccines induced very mild local reactions which were consistant with the presence of alum and the previoous administration of two doses of whole-cell DPT vaccine. A marked increase in specific antibodies to each pertussis component and in **pertussis toxin** neutralizing antibodies was observed after one dose of either acellular vaccines. All vaccines also acquired an excellent protective immunity against diptheria and tetnus, as assessed in vitro and in vivo.

L5 ANSWER 15 OF 19 MEDLINE DUPLICATE 4

ACCESSION NUMBER: 91034178 MEDLINE
DOCUMENT NUMBER: 91034178

TITLE: Engineering of genetically detoxified **pertussis toxin** analogs for development of a recombinant whooping cough vaccine.

AUTHOR: Loosmore S M; Zealey G R; Boux H A; Cockle S A; Radika K; Fahim R E; Zobrist G J; Yacoob R K; Chong P C; Yao F L; et al
CORPORATE SOURCE: Connaught Centre for Biotechnology Research, Willowdale, Ontario, Canada..

SOURCE: INFECTION AND IMMUNITY, (1990 Nov) 58 (11) 3653-62.
Journal code: GO7. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199102

AB **Pertussis toxin** (**PT**) is an important protective antigen in vaccines against whooping cough, and a genetically detoxified **PT** analog is the preferred form of the immunogen. Several amino acids of the S1 subunit were identified as functionally critical residues by site-directed mutagenesis, specifically, those at positions 9, 13, 26, 35, 41, 58, and 129. Eighty-three mutated **PT** operons were introduced into Bordetella parapertussis, and the resultant **toxin** analogs were screened for expression levels, enzymatic activity, residual toxicity, and antigenicity. While more than half of the mutants were found to be poorly secreted or assembled, the rest were fully assembled and most were highly **detoxified**. Single

mutations resulted in up to a 1,000-fold reduction in both toxic and enzymatic activities, while **PT** analogs with multiple mutations (Lys-9 Gly-129, Glu-58 Gly-129, and Lys-9 Glu-58 Gly-129) were 10(6)-fold detoxified. Operons coding for stable and nontoxic mutants shown to express a critical immunodominant protective epitope were returned to the chromosome of Bordetella pertussis by allelic exchange. In vivo analysis of the **toxin** analogs showed a dramatic reduction in histamine sensitization and lymphocytosis-promoting activities, paralleling the reduction in toxic activities. All mutants were protective in an intracerebral challenge test, and the Lys-9 Gly-129 analog was found to be significantly more immunogenic than the toxoid. **PT** analogs such as those described represent suitable components for the design of a recombinant whooping cough vaccine.

L5 ANSWER 16 OF 19 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 91175025 MEDLINE
DOCUMENT NUMBER: 91175025
TITLE: Gene replacement in Bordetella pertussis by transformation with linear DNA.
AUTHOR: Zealey G R; Loosmore S M; Yacoob R K; Cockle S A; Boux L J; Miller L D; Klein M H
CORPORATE SOURCE: Connaught Centre for Biotechnology Research, Willowdale, Ontario, Canada..
SOURCE: BIO/TECHNOLOGY, (1990 Nov) 8 (11) 1025-9. Journal code: AL1. ISSN: 0733-222X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: B
ENTRY MONTH: 199107
AB We replaced the wild-type TOX operon of Bordetella pertussis with in vitro **mutated**, **detoxified** alleles by electroporetic transformation using unmarked linear DNA. Uptake of DNA was selected by transient ampicillin resistance and two simultaneous recombination events resulted in gene-replacement at the natural locus with no integration of heterologous DNA. TOX alleles were stable without selection and recombinant strains secreted non-toxic, fully assembled, protective **pertussis toxin** (**PT**) analogues with kinetics similar to the parental vaccine strain under production-scale fermentation conditions. Strains generated in this way are suitable for the production of recombinant whole-cell or component whooping cough vaccines that require no chemical modification of **PT**.

L5 ANSWER 17 OF 19 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1990:365169 BIOSIS
DOCUMENT NUMBER: BR39:49645
TITLE: **DETOXIFICATION** OF **PERTUSSIS**

TOXIN BY **MUTATIONS** IN THE B OLIGOMER GENE.

AUTHOR(S): LOCHT C; FERON C; DEQUESNE G; DE WILDE M
CORPORATE SOURCE: SMITH KLINE BIOLOGICALS, 89 RUE DE L'INST., B-1330 RIXENSART, BELG.

SOURCE: 90TH ANNUAL MEETING OF THE AMERICAN SOCIETY FOR MICROBIOLOGY 1990, ANAHEIM, CALIFORNIA, USA, MAY 13-17, 1990. ABSTR ANNU MEET AM SOC MICROBIOL, (1990) 90 (0), 48.
CODEN: ASMACK. ISSN: 0094-8519.
DOCUMENT TYPE: Conference
FILE SEGMENT: BR; OLD
LANGUAGE: English

L5 ANSWER 18 OF 19 WPIDS
ACCESSION NUMBER: 1989-324080 [44] WPIDS
DOC. NO. NON-CPI: N89-246838
DOC. NO. CPI: C89-143525
TITLE: Vaccine contg. recombinant, detoxified Pasteurella multocida toxin - to protect against organisms producing osteolytic toxin.
DERWENT CLASS: B04 C03 D16 S03
INVENTOR(S): PETERSEN, S; TAEKKER, FOGED N; FOGED, N T; FOGED, T N; TEKKER, F
PATENT ASSIGNEE(S): (INTE-N) INTERVET INT BV; (STAT-N) STATENS VETERINAERE SERUMLABORATOTIUM; (NDKE) NORDISK DROGE & KEMIKALIE AS; (STAT-N) STATENS VETERIN SERUMLAB; (VETE-N) STATENS VETERINAERE SERU
COUNTRY COUNT: 23
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 8909617	A	891019	(8944)*	EN	121
RW: AT BE CH DE FR GB IT LU NL SE					
W: AU BE BR DK FI HU JP KR NO RO SU US					
AU 8935320	A	891103	(9003)		
EP 409895	A	910130	(9105)		
R: AT BE CH DE FR GB IT LI LU NL SE					
DK 9002308	A	900924	(9106)		
EP 409895	B1	940622	(9424)	EN	78
R: AT BE CH DE FR GB IT LI LU NL SE					
DE 68916424	E	940728	(9429)		
US 5369019	A	941129	(9502)		61
DK 169749	B	950213	(9511)		
IE 64033	B	950628	(9533)		
US 5885589	A	990323	(9919)		

APPLICATION DETAILS:

PATENT NO	KIND		APPLICATION	DATE
WO 8909617	A		WO 89-DK84	890411
EP 409895	A		EP 89-905073	890411
EP 409895	B1		EP 89-905073	890411
			WO 89-DK84	890411
DE 68916424	E		DE 89-616424	890411
			EP 89-905073	890411
			WO 89-DK84	890411
US 5369019	A		WO 89-DK84	890411
			US 90-582945	901012
DK 169749	B		WO 89-DK84	890411
			DK 90-2308	900924
IE 64033	B		IE 89-1151	890411
US 5885589	A	Cont of	WO 89-DK84	890411
		Cont of	US 90-582945	901012
		Div ex	US 94-293314	940822
			US 95-453141	950530

FILING DETAILS:

PATENT NO	KIND		PATENT NO
EP 409895	B1	Based on	WO 8909617
DE 68916424	E	Based on	EP 409895
		Based on	WO 8909617
US 5369019	A	Based on	WO 8909617
DK 169749	B	Previous Publ.	DK 9002308
US 5885589	A	Cont of	US 5369019

PRIORITY APPLN. INFO: DK 88-1995 880412; DK 90-2308 900924

AN 1989-324080 [44] WPIDS

AB WO 8909617 A UPAB: 19950918

Vaccine for immunising animals or humans against diseases caused by microorganisms producing an osteolytic toxin, comprises recombinant, immunogenic, detoxified Pasteurella multocida (P.m.) toxin, or toxin analogue, plus an acceptable carrier or vehicle. Also new are (1) DNA fragments encoding P.m. toxin (or analogues); (2) expression vectors contg. these fragments; (3) microorganisms contg. such vectors, and (4) monoclonal antibodies MAb; and their fragments against P.m. toxin.

The toxin, or analogue, is **detoxified** by heating; chemical treatment, **mutagenesis**; or by substitution, deletion, addn. or insertion of at least one amino acid (or base pair in the corresponding nucleic acid coding sequences). The specification includes the DNA sequence (and derived amino acid sequence) which encodes for the toxin (4380 bases).

USE/ADVANTAGE - The vaccines are used to protect against esp.

P.m. (which causes progressive atrophic rhinitis in pigs) but also e.g. Actinomyces viscosus and Bordetella **pertussis**. Recombinant **toxins** can be produced without culturing pathogenic organisms and in improved yields.
1/33
Dwg.1/33

ABEQ EP 409895 B UPAB: 19940803
A DNA fragment encoding a Pasteurella multocida toxin comprising an amino acid sequence as shown in Fig. 10(a)-(j) (in the specification) or encoding an immunogenic subsequence or analogue of said toxin.
Dwg.0/22

ABEQ US 5369019 A UPAB: 19950117
Recombinant DNA encodes the prodn. of a Pasteurella multicide toxin polypeptide. The nucleotide sequence of the cDNA and the aminoacid sequence of the polypeptide are defined.

Plasmids and expression vectors contg. the DNA are new. Host cells (e.g. Escherichia coli) are transformed with the plasmids and vectors and then propagated to produce and then reacted with HCHO or glutaraldehyde, or subjected to proteolytic enzymolysis, to give derivs. with much reduced toxicity.

USE/ADVANTAGE - The detoxified polypeptide derivs. are dispersed with the usual carriers and additives to provide vaccines against disease caused by the osteolytic Pasteurella multocida toxin. The vaccine is effective against porcine atrophic rhinitis.
Dwg.0/0

L5 ANSWER 19 OF 19 WPIDS COPYRIGHT 1999 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1989-186481 [26] WPIDS
CROSS REFERENCE: 96-041582 [05]
DOC. NO. CPI: C89-082452
TITLE: Immuno-protective, genetically-detoxified **pertussis toxin** and vaccine - with aminoacid substitution(s) or deletion(s) produced by site-directed mutagenesis of toxin gene.
DERWENT CLASS: B04 D16
INVENTOR(S): BOUX, H A; COCKLE, S A; KLEIN, M H; LOOSMORE, S M; ZEALEY, G R
PATENT ASSIGNEE(S): (CONN-N) CONNAUGHT LABS LTD; (CONN-N) CONNAUGHT LAB LTD
COUNTRY COUNT: 15
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
EP 322115	A	890628	(8926)*	EN	42
R: AT BE CH DE ES FR GB GR IT LI LU NL SE					
JP 02002383 A		900108	(9007)		

US 5085862	A	920204	(9208)		33
US 5221618	A	930622	(9326)		37
US 5244657	A	930914	(9338)		46
US 5332583	A	940726	(9429)		45
US 5358868	A	941025	(9442)		45
US 5433945	A	950718	(9534)		47
EP 322115	B1	960306	(9614)	EN	49
R: AT BE CH DE ES FR GB GR IT LI LU NL SE					
DE 3855072	G	960411	(9620)		
ES 2088778	T3	960916	(9643)		
JP 2714068	B2	980216	(9812)		37

APPLICATION DETAILS:

PATENT NO	KIND		APPLICATION	DATE
EP 322115	A		EP 88-311133	881124
JP 02002383	A		JP 88-297152	881124
US 5085862	A		US 88-275376	881123
US 5221618	A	Div ex	US 88-275376	881123
			US 91-767837	910930
US 5244657	A	CIP of	US 88-275376	881123
			US 90-589423	900928
US 5332583	A	CIP of	US 88-275376	881123
		Div ex	US 89-589423	890928
			US 91-788314	911105
US 5358868	A	CIP of	US 88-275376	881123
		Div ex	US 90-589423	900928
			US 91-788313	911105
US 5433945	A	CIP of	US 88-275376	881123
		Div ex	US 90-589423	900928
			US 92-979798	921120
EP 322115	B1		EP 88-311133	881124
DE 3855072	G		DE 88-3855072	881124
			EP 88-311133	881124
ES 2088778	T3		EP 88-311133	881124
JP 2714068	B2		JP 88-297152	881124

FILING DETAILS:

PATENT NO	KIND		PATENT NO
US 5221618	A	Div ex	US 5085862
US 5244657	A	CIP of	US 5085862
US 5332583	A	CIP of	US 5085862
		Div ex	US 5244657
US 5358868	A	CIP of	US 5085862
		Div ex	US 5244657
US 5433945	A	CIP of	US 5045862

Searcher : Shears 308-4994

		Div ex	US 5244657
DE 3855072	G	Based on	EP 322115
ES 2088778	T3	Based on	EP 322115
JP 2714068	B2	Previous Publ.	JP 02002383

PRIORITY APPLN. INFO: GB 87-27489 871124
AN 1989-186481 [26] WPIDS
CR 96-041582 [05]
AB EP 322115 A UPAB: 19960212
An immunoprotective, genetically-**detoxified mutant** of **pertussis toxin** is new. Vaccine against Bordetella pertussis comprises an effective amt. of the mutant, or its toxoid, and an acceptable carrier. Conjugate vaccine comprises the mutant as carrier protein for a hapten, polysaccharide or polypeptide. New strains of Bordetells pertussis are characterised by either (i) the absence of the toxin operon and foreign DNA and by the ability to be grown in the absence of antibiotics to produce B. pertussis antigens free of **pertussis toxin**; or
(ii) the toxin operon having been replaced by a mutant gene formed by site-directed mutagenesis of at least one specific amino acid residue responsible for **pertussis toxin** toxicity. Native Bordetella pertussis 10536 TOX operon is new having a given nucleotide sequence and structural gene translation.

ADVANTAGE - Residual toxicity is 1% or less, pref. less than 0.5% of that of the native toxin. Genetic detoxification avoids the problems of chemical detoxification using e.g. formaldehyde, glutaraldehyde or H2O2, i.e. obtaining a balance between sufficient detoxification and loss of potency.

0/20
Dwg.0/20
ABEQ US 5085862 A UPAB: 19930923
Immunoprotective genetically **detoxified mutant** of pertussis holotoxin is formed by genetic modification of the A portion (S1 subunit) and/or B portion of the holotoxin.

Pref. a single amino acid in the native holotoxin is removed or replaced e.g. glu-129 is removed and opt. replaced by gly, or arg-58 is replaced by glu, etc. Mutant has residual toxicity, less than 0.5% of native toxin.

ADVANTAGE - Has decreased histamine sensitivity in a vaccine against Bordetella pertussis.
ABEQ US 5221618 A UPAB: 19931116
Strain of Bordetella capable of expressing an immunoprotective genetically-**detoxified mutant** of **pertussis** holotoxin. **Toxin** operon has been replaced by a mutant operon formed by mutagenesis of a nucleotide sequence encoding at least one specific aminoacid residue which contributes to **pertussis toxin** toxicity.

Also claimed is a method of producing an immunoprotective, genetically-**detoxified** pertussis holotoxin **mutant**

USE/ADVANTAGE - As a vaccine against pertussis.
Dwg.0/10

ABEQ US 5244657 A UPAB: 19931123
Immunoprotective genetically-**detoxified mutant** of **pertussis** halo-**toxin** has a single amino acid in its S1 sub-unit of the native form replaced, i.e. arg9 by lys9.
Mutant has residual toxicity less than 0.5% of native toxic. Prodn. comprises site-directed mutagenesis of native **pertussis toxin** gene. Mutant has decreased histamine sensitivity activity.
USE - In prepn. of safe, immunogenic and efficacious vaccine for protection against pertussis.
Dwg.0/15

ABEQ US 5332583 A UPAB: 19940907
Vaccine against Bordetella pertussis comprises a mutant of pertussis holotoxin (where at least one amino acid is removed or replaced) and at least one other pertussis antigen e.g. agglutinogens, FHA or 69 kD membrane protein.
ADVANTAGE - Vaccine is safe and effective.
Dwg.0/29

ABEQ US 5358868 A UPAB: 19941212
Strain of Bordetella has the toxin operon replaced by a mutant gene formed by site-directed mutagenesis of a sequence encoding the S1 and S3 subunit of pertussis holotoxin. Has ATCC Nos. 53833, 53834, 53836, 53837, 53974, 53975 or 53976.
USE/ADVANTAGE - Prepn. of a vaccine against pertussis. Vaccine is safe.
Dwg.0/29

ABEQ US 5433945 A UPAB: 19950904
Immunoprotective genetically-**detoxified mutant** of pertussis holotoxin has multiple amino acids in the native toxin replaced or removed. Specific examples include Arg-58 and Gly-129 replaced by Glu-58 and Gly-129, and Arg-9 and Glu-129 replaced by Ly's-9 and Gly-129 in the SI subunit. Mutants have a residual toxicity of less than 0.5%.
USE/ADVANTAGE - Used as a vaccine against pertussis. Retains immunological properties without having undesirable side effects. decreased histamine sensitivity.
Dwg.0/15

ABEQ EP 322115 B UPAB: 19960405
A mutant pertussis holotoxin obtained by expression of a tox operon encoding the holotoxin which has been mutated by site-directed mutagenesis of at least one codon encoding at least one functional amino acid within native pertussis holotoxin including at least one of (A1) ARG9, ARG13 and GLU129, to effect removal or replacement of said at least one functional amino acid and to genetically detoxify said holotoxin to a residual toxicity of 1% or less while retaining immunoprotective properties.

Dwg.0/10

=> d his l6-; d 1-6 ibib abs

(FILE 'CAPLUS, MEDLINE, BIOSIS, EMBASE, TOXLIT, TOXLINE, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, CABA, AGRICOLA' ENTERED AT 15:23:45 ON 27 JUL 1999)

Author(s)

```
L6          74 S BARCHFELD G?/AU
L7         580 S (DELGIUDICE G? OR DEL GIUDICE G?)/AU
L8        1744 S RAPPUOLI R?/AU
L9           2 S L6 AND L7 AND L8
L10          7 S L6 AND (L7 OR L8)
L11         46 S L7 AND L8
L12          7 S L11 AND L1
L13         12 S L9 OR L10 OR L12
L14          6 DUP REM L13 (6 DUPLICATES REMOVED)
```

L14 ANSWER 1 OF 6 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 1

ACCESSION NUMBER: 1999:164424 CAPLUS
DOCUMENT NUMBER: 131:17638
TITLE: The adjuvants MF59 and LT-K63 enhance the mucosal and systemic immunogenicity of subunit influenza vaccine administered intranasally in mice
AUTHOR(S): **Barchfeld, G. L.**; Hessler, A. L.; Chen, M.; Pizza, M.; **Rappuoli, R.**; Van Nest, G. A.
CORPORATE SOURCE: Chiron Corporation, Emeryville, CA, 94608-2916, USA
SOURCE: Vaccine (1999), 17(7-8), 695-704
CODEN: VACCDE; ISSN: 0264-410X
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Com. influenza vaccines generate serum antibody, but not local IgA. Influenza vaccines that induce both serum and secretory antibody are more likely to protect against infection and disease progression. The adjuvants MF59 and LT-K63 were tested i.m. and intranasally with subunit HA. In naive mice, intranasal adjuvant effect was more apparent when included with the first than second immunization. In previously infected mice, intranasal adjuvants had little effect on serum antibodies and were most effective for nasal antibodies after the second immunization. Overall, both adjuvants enhanced anti-HA IgA and IgG by intranasal vaccination whereas, by i.m. vaccination, they only enhanced serum IgG.

L14 ANSWER 2 OF 6 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 2

ACCESSION NUMBER: 1998:672490 CAPLUS
DOCUMENT NUMBER: 129:289177

TITLE: Detoxified mutants of bacterial **ADP-ribosylating toxins** as parenteral adjuvants

INVENTOR(S): **Barchfeld, Gail; Del Giudice, Giuseppe; Rappuoli, Rino**

PATENT ASSIGNEE(S): Chiron Corporation, USA

SOURCE: PCT Int. Appl., 51 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9842375	A1	19981001	WO 98-US5454	19980319
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9865713	A1	19981020	AU 98-65713	19980319
PRIORITY APPLN. INFO.:			US 97-41227	19970321
			US 98-44696	19980318
			WO 98-US5454	19980319

AB The present invention provides parenteral adjuvants comprising detoxified mutants of bacterial **ADP-ribosylating toxins**, esp. **pertussis toxin** (**PT**), **cholera toxin** (**CT**), and Escherichia **coli**-derived heat-labile **toxin** (**LT**). The immune adjuvant includes LT-K63, LT-R72, CT-S109 and PT-K9/G129. LT-K63 was prepd. as parenteral adjuvant for vaccine comprising herpes simplex virus type 2 gD antigen, influenza hemagglutinin, and HIV p24 gag.

L14 ANSWER 3 OF 6 SCISEARCH COPYRIGHT 1999 ISI (R)

ACCESSION NUMBER: 1998:308436 SCISEARCH

THE GENUINE ARTICLE: ZH131

TITLE: Mucosal adjuvanticity and immunogenicity of LTR72, a novel mutant of Escherichia coli heat-labile enterotoxin with partial knockout of ADP-ribosyltransferase activity

AUTHOR: Giuliani M M; **DelGiudice G**; Giannelli V; Dougan G; Douce G; **Rappuoli R (Reprint)**; Pizza M

Searcher : Shears 308-4994

CORPORATE SOURCE: CHIRON SPA, IRIS, VIA FIORENTINA 1, I-53100 SIENA, ITALY (Reprint); CHIRON SPA, IRIS, I-53100 SIENA, ITALY; UNIV LONDON IMPERIAL COLL SCI TECHNOL & MED, DEPT BIOCHEM, LONDON SW7 2AY, ENGLAND
COUNTRY OF AUTHOR: ITALY; ENGLAND
SOURCE: JOURNAL OF EXPERIMENTAL MEDICINE, (6 APR 1998) Vol. 187, No. 7, pp. 1123-1132.
Publisher: ROCKEFELLER UNIV PRESS, 1114 FIRST AVE, 4TH FL, NEW YORK, NY 10021.
ISSN: 0022-1007.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 58
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Heat-labile Escherichia coli enterotoxin (LT) has the innate property of being a strong mucosal immunogen and adjuvant. In the attempt to reduce toxicity and maintain the useful immunological properties, several LT mutants have been produced. Some of these are promising mucosal adjuvants. However, so far, only those that were still toxic maintained full adjuvanticity. In this paper we describe a novel LT mutant with greatly reduced toxicity that maintains most of the adjuvanticity. The new mutant (LTR72), that contains a substitution Ala --> Arg in position 72 of the A subunit, showed only 0.60% of the LT enzymatic activity, was 100,000-fold less toxic than wild-type LT in Y1 cells in vitro, and was at least 20 times less effective than wild-type LT in the rabbit ileal loop assay in vivo. At a dose of 1 mu g, LTR72 exhibited a mucosal adjuvanticity, similar to that observed with wild-type LT, better than that induced by the nontoxic, enzymatically inactive LTK63 mutant, and much greater than that of the recombinant B subunit. This trend was consistent for both the amounts and kinetics of the antibody induced, and priming of antigen-specific T lymphocytes. The data suggest that the innate high adjuvanticity of LT derives from the independent contribution of the nontoxic AB complex and the enzymatic activity. LTR72 optimizes the use of both properties: the enzymatic activity for which traces are enough, and the nontoxic AB complex, the effect of which is dose dependent. In fact, in dose-response experiments in mice, 20 mu g of LTR72 were a stronger mucosal adjuvant than wild-typs LT. This suggests LTR72 may be an excellent candidate to be tested in clinical trials.

L14 ANSWER 4 OF 6 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1998:673563 CAPLUS
DOCUMENT NUMBER: 130:79917
TITLE: LT and CT mutants as mucosal adjuvants
AUTHOR(S): **Del Giudice, Giuseppe**; Pizza, Mariagrazia; **Rappuoli, Rino**
CORPORATE SOURCE: Chiron SpA Res. Cent., IRIS, Siena, 53100, Italy
Searcher : Shears 308-4994

SOURCE: Mol. Aspects Med. (1998), 19(1), 37-46, 47-70
CODEN: MAMED5; ISSN: 0098-2997
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review with approx. 275 refs. about **cholera toxin** and Escherishia **coli** heat-labile **toxin** as mucosal adjuvants and immunogens and their possible use a mucosally delivered vaccines.

L14 ANSWER 5 OF 6 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1998:673548 CAPLUS
DOCUMENT NUMBER: 130:79916
TITLE: PT mutants as vaccines against pertussis
AUTHOR(S): **Del Giudice, Giuseppe**; Pizza, Mariagrazia; **Rappuoli, Rino**
CORPORATE SOURCE: Chiron SpA Res. Cent., IRIS, Siena, 53100, Italy
SOURCE: Mol. Aspects Med. (1998), 19(1), 27-36, 47-70
CODEN: MAMED5; ISSN: 0098-2997
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review with more than 100 refs. Topics discussed include whole cell pertussis vaccine; B. pertussis antigens for acellular pertussis vaccines; genetically detoxified acellular pertussis vaccines; improved immunogenicity and efficacy of genetically detoxified recombinant pertussis vaccine;.

L14 ANSWER 6 OF 6 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 3
ACCESSION NUMBER: 1998:419520 CAPLUS
DOCUMENT NUMBER: 129:197743
TITLE: Immunogenicity and adjuvanticity of partially or completely detoxified LT derivatives
AUTHOR(S): Giuliani, M. M.; **Del Giudice, G.**; Douce, G.; Dougan, G.; **Rappuoli, R.**; Pizza, M.
CORPORATE SOURCE: IRIS, Chiron Vaccines Immunobiological Research Institute in Siena, Italy
SOURCE: Zentralbl. Bakteriol., Suppl. (1997), 29(Bacterial Protein Toxins), 458-460
CODEN: ZBASE2; ISSN: 0941-018X
PUBLISHER: Gustav Fischer Verlag
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Heat-labile enterotoxin (**LT**) from the enterotoxigenic Escherichia coli is an **ADP-ribosylating toxin**. It is a strong immunogen, but its toxicity has precluded its use in the mucosally delivered vaccines. Here, the authors describe a new mutant in the A subunit of LT, LTR72 (Ala72

to Arg) which retains a residual but very low toxicity. They compare the immunogenicity and adjuvanticity of LTR72 with those of the fully active wild-type LT, the non-toxic LTK63 mutant, and the recombinant LTB to exploit the role of the A subunit and of ADP-ribosylating activity on mucosal immunogenicity and adjuvanticity.

=> fil hom

FILE 'HOME' ENTERED AT 15:27:25 ON 27 JUL 1999